

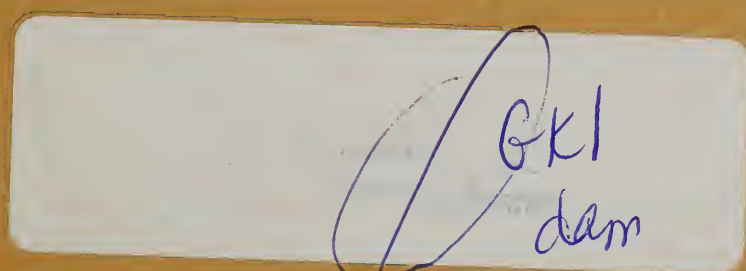
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PROCEEDINGS OF THE 1998 SUGAR PROCESSING RESEARCH CONFERENCE



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MARCH 22-25, 1998
SAVANNAH, GEORGIA, USA



**PROCEEDINGS OF THE
1998 SUGAR PROCESSING
RESEARCH CONFERENCE**

**MARCH 22-25, 1998
SAVANNAH, GEORGIA, USA**

**Sponsored by
Sugar Processing Research Institute, Inc.**

December, 1998

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PREFACE

The 1998 Sugar Processing Research Conference is one of a series of Conferences held in alternate years to provide a forum for exchange of information among technical leaders of the sugar industry and to report on new and noteworthy developments. The Conference is sponsored by Sugar Processing Research Institute, Inc. (S.P.R.I.). The Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, contributed in kind to the organization of the Conference.

The program for this Conference was arranged by Margaret A. Clarke. The Conference Coordinator was Shirley T. Saucier. These Proceedings were edited by Mary An Godshall with Editorial Assistant Janell D. Becker.

The series, Proceedings of the Sugar Processing Research Conference, of which this is the ninth issue, continues the Proceedings of the Technical Sessions on Cane Sugar Refining Research, which was published every other year from 1964 to 1980. For individual copies of this volume as well as back issues of the former series as long as the supply lasts, write to Sugar Processing Research Institute, Inc., 1100 Robert E. Lee Blvd., New Orleans, LA 70124. Before 1986, Proceedings were published by the Agricultural Research Service, U.S. Department of Agriculture. Since 1988, Proceedings have been published by the Sugar Processing Research Institute, Inc.

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This volume is
dedicated to the memory of
Margaret A. Clarke
1942 - 1998

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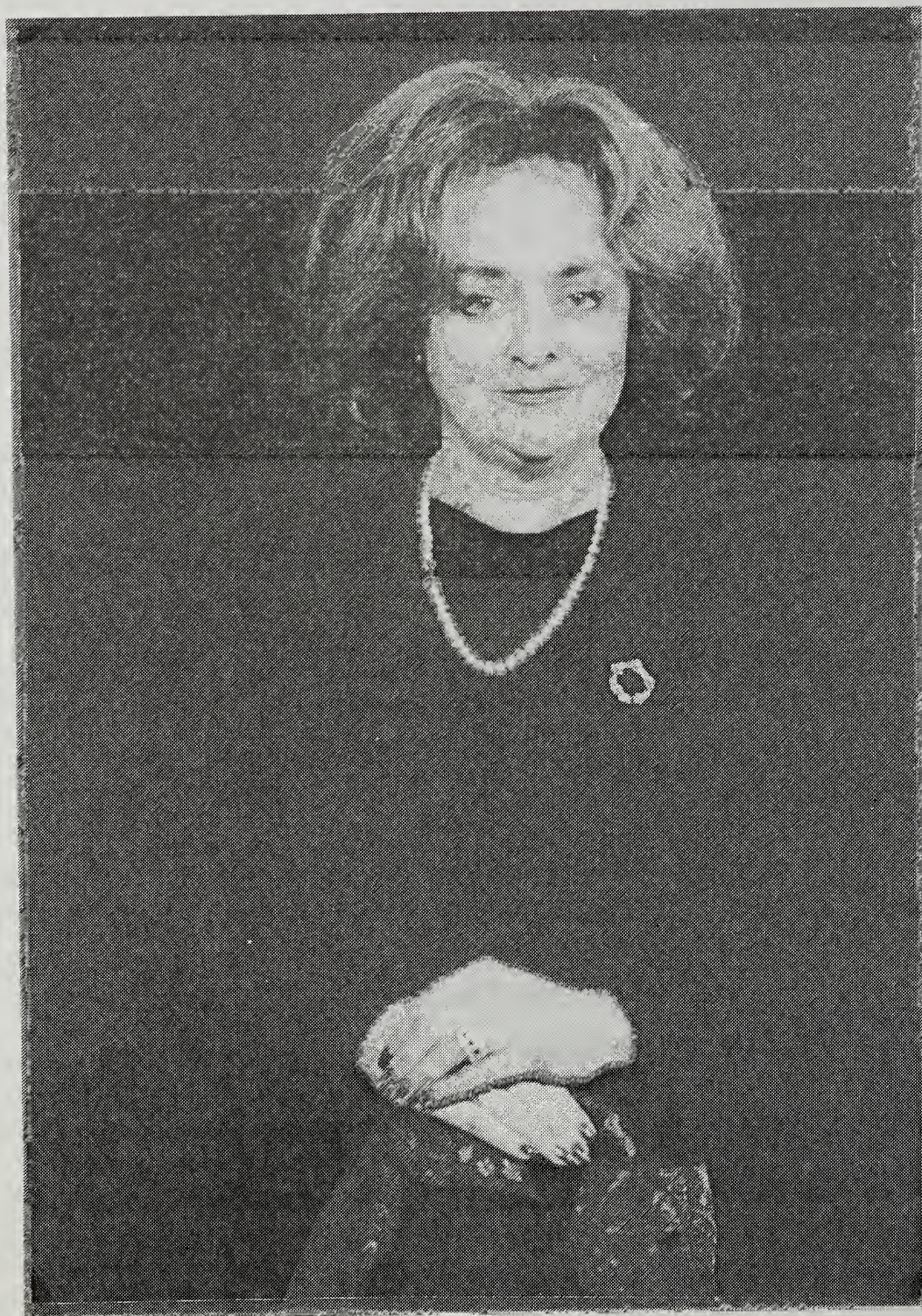
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In Memoriam

Margaret A. Clarke

1942 - 1998



Margaret A. Clarke

1942 - 1998

The worldwide sugar industry was shocked and saddened by Margaret Clarke's untimely death on June 18, 1998.

Margaret Clarke was born May 8, 1942, in Belfast, Ireland. When she was a young child, her parents emigrated to Canada. She received her B.Sc. at the University of Western Ontario in 1963 and her Ph.D in 1970 in physical inorganic chemistry at Tulane University in New Orleans. In 1980, she received an M.B.A. from Loyola University, New Orleans.

She left the harsh winters of Canada for the warmth of Louisiana and made New Orleans her home for the rest of her life, although the entire sugar world was her stage.

She began her employment with the Cane Sugar Refining Research Project in 1969 as a research chemist and later was named the Managing Director in 1981, when it became the Sugar Processing Research Institute.

During her tenure at S.P.R.I., she continued the work begun by Dr. Frank G. Carpenter, the first Director of the Cane Sugar Refining Research Project, expanding the scope of endeavor from cane sugar refining to all aspects of sugar production, including beet production. Under her direction, the number of industrial sponsors of S.P.R.I. expanded to over 40 members.

Margaret Clarke was a tireless worker for the cause of sugar, traveling and lecturing around the world. She published over 200 papers, contributed many book and encyclopedia articles and organized or contributed to innumerable meetings. She served on the editorial boards of *Sugar Industry Abstracts*, *Sugar Technology Reviews* and *Seminars in Food Analysis*. She organized the Conferences on Sugar Processing Research, of which the present volume is part. She instituted the S.P.R.I. Workshops, a diverse series devoted to timely aspects of sugar processing or analysis. She was also responsible for the S.P.R.I. Science Award and the S.P.R.I. Industrial Technology Award.

In 1985, she began the New Orleans Carbohydrate Symposium, a series of Gordon conference style, small informal meetings attended, by invitation only, by the highest caliber of carbohydrate chemists in the world. The thirteenth conference was held in April, 1998.

In her last months, while ill, she organized three major meetings - the 13th New Orleans Carbohydrate Symposium, the Sugar Processing Research Conference, and the S.P.R.I. Workshop on Sugar Around the World. She also attended the Sugar Industry Technologists meeting and the 22nd Session of ICUMSA.

She was tireless and devoted, and her loss is keenly felt by many around the world.

1998 S.P.R.I. Science Award

Markwart Kunz



Markwart Kunz, born in Lower Saxony, Germany in 1948, obtained his undergraduate degree (Dipl.-Ing.) at the Technical University of Braunschweig, where he continued work on his doctorate, with Prof. E. Reinefeld, at the Institute of Agricultural Technology and the Sugar Industry. In 1977, he received the Ph.D. (Dr.-Ing.) with his thesis title "On the reactivity of invert sugar components during carbonylamino reactions in technical sugar juices", and began work at Südzucker AG, as Production Engineer, Regensburg Refinery. In 1981, he moved to the Südzucker Head Office, in Mannheim, as Managing Advisor to the Board with respect to Sugar Chemistry and Technology, where he remained until 1986. During this time, he established a research group at the Sugar Institute at Braunschweig Technical University, on "Exploitation of sucrose chemistry toward novel, industrially viable products" which resulted in 15 Ph.D. dissertations and many papers, most on novel polymers e.g. polyvinylsaccharides and tensides, until the Braunschweig group closed in 1996.

In 1986, Dr. Kunz became Managing Director of the PalatinitTM Division of Südzucker, in charge of construction of the new PalatinitTM plant at Offstein/Pfalz, and then, in 1992, took the position of Research Director, Central Research Department of Südzucker AG, Offstein/Pfalz, where he is today. He maintains his academic connection as Guest Lecturer on special topics in carbohydrates at the Chemistry Faculty of the Technical University of Darmstadt.

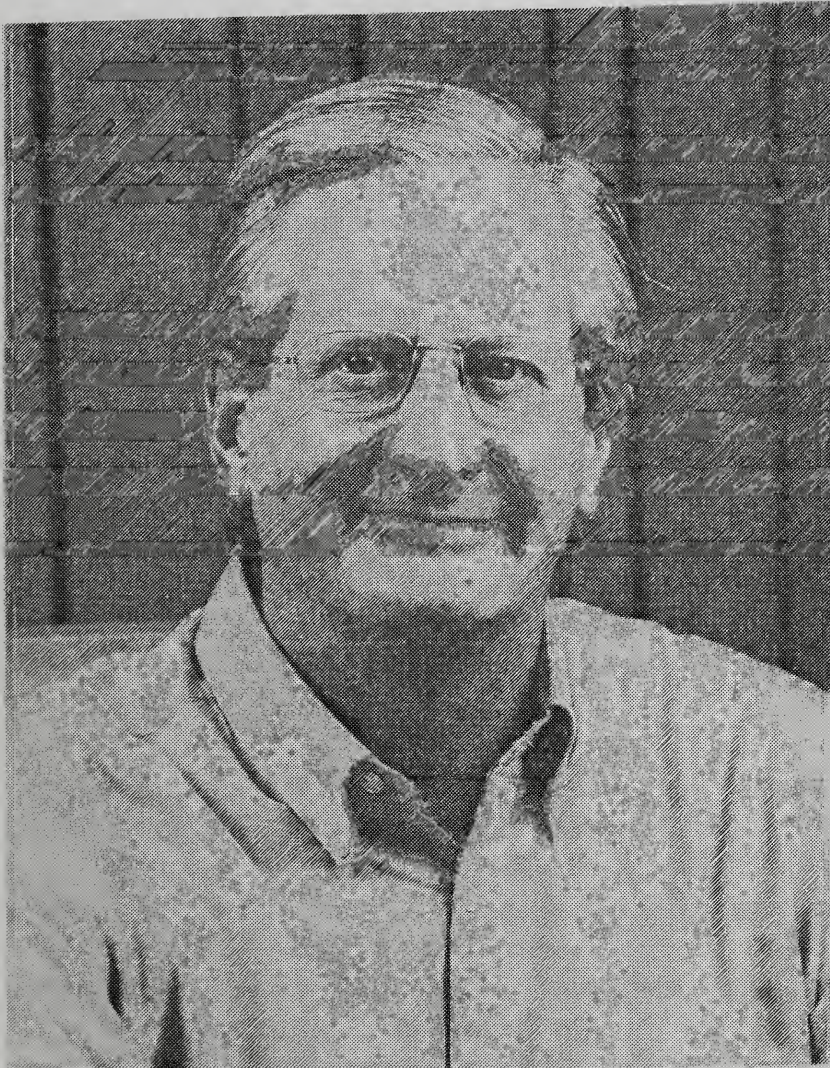
Dr. Kunz has published many papers on sucrose, sucrose reactions, sucrose polymers and derivatives. He holds 23 patents on these subjects. He is a member of the German Chemical Society, Max Planck Society, Verein Deutscher Zuckertechniker, WSRO and CITS Scientific Committees, German National Committee to ICUMSA and the German Research Council of the Food Industry.

He is interested in history, music, and philosophy.

Dr. Kunz's keynote lecture at the Conference was entitled, "Sucrose - Raw Material for Chemistry and Biochemistry".

1998 S.P.R.I. Industrial Technology Award

Peter Rein



Peter Rein, born and schooled in South Africa, attended the University of Cape Town on a DeBeers scholarship. After graduating with top honours, he finished his M.Sc. in 1967 and worked on synthetic diamonds at DeBeers research labs until 1970. Then he moved to Hulett (now Tongaat-Hulett Sugars Ltd.) and began his important work on diffusion, in cooperation with the University of Natal; the dissertation for his Ph.D. (awarded 1973) was on mechanisms of diffusion. He became Engineer in Charge of the first Hulett-designed diffuser, at Amatikulu, the success of which engendered widespread adoption of diffusers.

Dr. Rein's significant developments in continuous pan boiling followed his success in diffusion. The new Felixton factory implemented his work in the mid-80's with continuous pan boiling on every grade of sugar; this pan design has been widely adopted. Other innovations and projects led by Peter, as Head of the Sugar Technology Department at Hulett's, include expansion of the Hulett Refinery ion-exchange decolorization plant, use of pan stirrers to

improve white boiling, innovations in automated white boiling systems and developments in syrup clarification. He has published some thirty technical papers and several patents.

Dr. Rein is at present a Director of Tongaat-Hulett Sugar, and is Head of the Technical Management Department. He has served on the National Council of the Institution of Chemical Engineers in South Africa. He is strongly interested in training of young technologists, and chairs a Committee to guide an Industrial Training Centre. He has served as President of the South African Sugar Technologists' Association, Congress organizer and Executive Committee member for Sugar Industry Technologists, and Chairman of the Technical Committee of the International Society of Sugar Cane Technologists. He is Vice-Chairman of the Board of Control of the Sugar Milling Research Institute.

Peter and his wife Kath, who is also a scientist, include golf, music and birdwatching among their leisure pursuits. They have three children, all professionally qualified.

THE S.P.R.I. AWARDS

The S.P.R.I. Science Award

The S.P.R.I. Science Award was instituted in 1986. The purpose of the award is to promote science and technology in sucrose processing and production. The award is offered biennially to an outstanding scientist whose research and development accomplishments have been distinguished by their originality and by their contribution to the sucrose processing and production industry.

The winner is chosen by an award committee consisting of three members, two from a university and/or sugar research institute, and one from a sugar processing and production company, who serves as the committee chair.

The award consists of a cash honorarium, a plaque and travel expenses.

Winners of the S.P.R.I. Science Award and Lectures

- 1998 Markwart Kunz, Südzucker AG, Mannheim/Ochsenfurt, Germany
"Sucrose - Raw Material for Chemistry and Biochemistry"
- 1996 Pascal A. Christodoulou, Hellenic Sugar Industry, Thessaloniki, Greece
"Energy Economy Optimization in the Separation Processes of Sucrose-Water and Non-Sugars"
- 1994 Frieder W. Lichtenthaler, Institute of Organic Chemistry, Technical University of Darmstadt, Germany
"Computer Simulation of Chemical and Biological Properties of Sucrose, the Cyclodextrins and Amylose"
- 1992 Riaz Khan, Poly-Bios, Trieste, Italy
"Chemical and Enzymic Transformations of Sucrose"
- 1990 Giorgio Mantovani, University of Ferrara, Italy
"Growth and Morphology of Sucrose Crystal"
- 1988 Leslie Hough, King's College London, (KQC), Department of Chemistry, Kensington, London
"Sucrose, Sweetness and Sucralose"
- 1986 Andrew VanHook, Chemistry Department, Holy Cross College, Worcester, Massachusetts
"Events in Sugar Crystallization"

S.P.R.I. Industrial Technology Award

The S.P.R.I. Industrial Technology Award was instituted in 1998 for the purpose of promoting technology in sugar processing and production. It is to be offered biennially to one outstanding individual whose contribution to the industry in the development of technology, or implementation of technology, has been distinguished by its originality and the resulting benefit to the industry.

The award winner is chosen by an award committee consisting of five individuals, two from a sugar research institute and three from sugar industry companies.

The award consists of a cash honorarium, a plaque and reasonable travel expenses to the recipient.

The first winner of the S.P.R.I. Industrial Technology Award was Pater Rein of Tongaat-Hulett Sugar in South Africa.

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Hyatt Regency Savannah Hotel

Savannah, Georgia

March 22-25, 1998

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S.P.R.I. SCIENCE AWARD KEYNOTE SPEECH

SUCROSE - RAW MATERIAL FOR CHEMISTRY AND BIOCHEMISTRY

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With its vast availability in an exceptionally pure condition, ease of transportation and storage in bulk quantities, sucrose may be regarded as a most suitable raw material for processing to value-added products.

The usually clear distinction between food and chemicals in the sense that chemicals are used only for non-food applications is not universally applicable, as for example starch and starch-derived products are used extensively not only in the food industry but also in paper and plastics. Therefore this paper will discuss only chemical or biochemical reactions including fermentations based on sugars.

The suitability of sucrose as raw material depends on:

- the gross economic conditions in the country in which you want to use it,
- the relative cost of a variety of different agricultural crops, for instance the competition between corn and beet,
- the relative cost of agricultural crops compared to crude oil and the special attributes the product in question should have.

Sucrose or its constituents glucose and fructose can be put to different material uses:

- as fermentation feed stock mainly accompanied by a destruction of the carbohydrate structure,
- and by chemical or biochemical modifications accompanied by destruction, conservation or reorganization of the carbohydrate structure.

Fermentation Products

Some fermentation products, especially citric and lactic acids, both based on sugar or glucose syrups depending on the regional cost and ecological conditions, are produced at nearly a million tonnes per year in some countries. Besides that, ethanol and acetic acid are produced in competition to the identical petrochemical products mainly because of political or ethical reasons.

There can be chances for new large scale fermentation products in countries like Germany only if:

- these are very special products with special application profiles not possible to cover by other cheaper products based on other raw materials,
- and if the product has a very high performance.

Chemical/Biochemical Modifications

a) Degradation of the carbohydrate structure

Probably one of the most famous products of the degradation of sugar is the molecule 5-hydroxymethyl furfural (HMF) which is in fact the reduction of a carbohydrate to a petrochemical like compound. HMF can be used in a variety of chemical reactions leading to very promising products. However, cost calculations, under best case conditions for a production scenario reaching about 10,000 metric tonnes per year showed the minimum production cost for HMF to be about two dollars a kilogram. Taking into account the additional cost to transform HMF, for instance into the corresponding dicarboxylic acid which could theoretically replace terephthalic acid in the production of aramide fibres, HMF is still not competitive, as terephthalic acid is much cheaper.

A catalytic hydrocracking reaction developed by BASF leads to dihydroxy alkanes, especially propanediol from sucrose. Propane diol production today is, however, integrated into petrochemical networks with byproduct utilization so that a stand-alone production based on sucrose is not competitive. In fact, the degradation of carbohydrates to molecules such as HMF, ethanol and propanediol to replace identical petrochemicals does not seem to be advisable in view of chemical and economical reasons.

Thus there will be chances for new products based on renewable resources like sucrose only if as much as possible of the sucrose used is recovered in the final molecule. This restricts the scope of reactions to be used to those giving new products while maintaining or reorganizing the carbohydrate structure.

b) Conservation of the carbohydrate structure

The best known examples of chemical reactions applied to sucrose while maintaining its structure are especially the production of polyether polyols and of a variety of sucrose esters.

As far as direct sucrose derivatives are concerned, small market niches for products within a price range between three DM and two hundred DM per kg are possible and to some extent being realized. But a bright future in that area does not seem to be very probable because of the lack of new applications, despite the intensive research done in this field for decades.

Sugar has eight hydroxyl groups but no specific chemical functionality suitable for selective reactions. Therefore the task is to introduce specifically new functionalities into the sucrose molecule.

A variety of hydroxy carboxylic acids are on the market including acids with one, two, four or five hydroxyl groups and one, two or three carboxylic groups. Though the oxidation of carbohydrates using e.g. platinum catalysts is well known, the application of this method to sucrose resulted in a complex mixture of unreacted sucrose, mono-, di- and tri- carboxylic acids and degradation products. The solution to this problem was a continuous oxidation process with product removal by electrodialyses. It is a very promising reaction though we still have some problems with the catalysts available. They are too expensive for industrial use and call for further research in catalyst development.

Besides that we also developed an interesting application for such sucrose derivatives. Simple acetylation of these sugar acids results in a promising booster-builder system which could be used in household surfactant mixtures.

It generates peracetate with sodium perborate or percarbonate to the same extent as with standard TAED (*N*, *N*, *N'*, *N'* - tetracetythylenediamine) system, an important fact for a booster and at the same time it has a better calcium complexing capacity than TAED. Thus using this system in surfactant formulations one can save on other anti-scaling agents such as citric acid or polyacrylates.

c) Reorganization of the carbohydrate structure

Chemical and biochemical reactions resulting in a reorganization of the sucrose molecule while maintaining the general carbohydrate structure seem to be the most promising reactions for product diversification in the sugar industry. This way it is possible to produce a variety of other carbohydrates starting from sucrose:

- monosaccharides by sucrose hydrolysis,
- sucrose isomers,
- higher oligosaccharides and
- polymers.

Best known is the splitting of sucrose to mixtures of glucose and fructose, the so-called invert sugar syrups used as sweeteners and for the production of fructose, sorbitol and mannitol. Südzucker makes use of both chemical and biochemical catalysis for the hydrolysis of sucrose.

With the background of this experience Südzucker started to gain know-how in other new technologies e.g. industrial scale chromatographic separation systems so that Südzucker became probably the first company to crystallize fructose on a large industrial scale using just water as solvent.

It was at about the same time that the company which had till then been a regional enterprise based only on sucrose production decided to extend its activities into biotechnology. In the mid-fifties, Professor Weidenhagen, a member of the staff of Südzucker, had detected a microorganism able to isomerize sucrose into isomaltulose (palatinose). Other similar isomerization reactions of sucrose leading to trehalulose or leucrose were found later by others.

Besides its use as a sweetener, palatinose is presently being employed as:

- intermediate, specially for the production of isomalt or for
- other potential applications to be discussed later and
- as reducing agent for textile dyes.

The most important field of application for isomalt is in a variety of foods as a sugar replacer. Besides that, it is also used as a plasticizer for melamine resins, as green bond and as adhesive for the production of special glasses.

Coming back to biotechnology we can discuss other possibilities offered by the unique sucrose molecule. The bond between glucose and fructose in sucrose has such a high energy content, that sucrose can be used as the donor to transfer either glucosyl or fructosyl moieties to other molecules to produce a variety of higher oligomers besides isomerized products such as palatinose or leucrose.

The best known example is the formation of fructooligosaccharides by the transfer of the fructose moiety from one sucrose molecule to another. This reaction is applied both in Europe and Japan to produce several thousand tonnes per year. Südzucker produces comparable products by hydrolysis of inulin.

The formation of high molecular weight polymers, especially dextran and levan has been known for decades. Within the last years it was found that unbranched or nearly unbranched glucan and fructan polymers similar to amylose and inulin could be synthesized from sucrose.

Isomers and oligomers with a high market potential in a price range between one and three dollars per kilogram have been successfully introduced to the market; new products seem to be in the pipeline. In case of polymeric products, further research and development to improve production technology and application profiles is still needed.

This lecture should also give an outlook on some reactions which are further away from application today but may find industrial use in say ten years or so from now.

New chances for chemical reactions with sucrose are to be found especially in reaction sequences such as isomerization of the non-reducing sucrose to a reducing carbohydrate followed by a chemical modification. This can help in introducing specific functionalities such as carboxylic or amino groups or hydrophobic functions.

The introduction of an amino group to reducing sugars is done easily in aqueous solution by reductive amination using ammonia, hydrazine or other suitable amination reagents and classical catalysts like Raney type nickel. This reaction was applied to palatinose to yield interesting products with promising application profiles.

CONCLUSIONS

- Sugar is a basic material, produced with an exceptionally high purity and on a large industrial scale using technology comparable to the chemical industry.
- Starting with sugar and using chemical and biochemical reactions, various products are being produced and sold today.
- The application of more advanced biochemical reactions will give sugar a new chance as raw material to produce a variety of products for different markets.

S.P.R.I.

- Further research and development work, in close cooperation between academia, scientific institutes and industry is necessary.
- Sugar companies are ideally suited to exploit the chances sugar affords them as raw material for new products but they will have to adapt their philosophy to include sophisticated unit processes along with their standard unit operations in their technology.

LIME REDUCTION IN JUICE PURIFICATION

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ABSTRACT

Although British Sugar has reduced its consumption of lime for purification of beet raw juice by about 50% over the last 15 years, the costs of using lime are still significant. Costs are incurred in purchase and transport of lime and for coke to burn the lime in conventional kilns. Offset against these is the benefit of the valuable used lime now sold as a convenient high dry substance product. This paper looks at some experimental work carried out by British Sugar to develop practical means of further reducing lime consumption by the factories.

INTRODUCTION

In an earlier paper some experimental work carried out within British Sugar using a small scale continuous carbonation apparatus was described (15). As a consequence of the work contained in that study and as a result of the persistent efforts of the factory staff the average use of lime in juice purification has fallen to 1.2% CaO (1996/7 campaign) from an average level of above 2% CaO on beet. No changes were made to the chemistry of the process and very little to the plant itself. Some changes were made to the way in which the process is controlled particularly in the introduction of Statistical Process Control into various areas of the factory in order to reduce the variability of the process.

In effect the savings in lime and coke were made by attention to detail and bringing the plant up to its full potential.

It would still be advantageous to be able to reduce further the consumption of lime to process. From an environmental point of view limestone quarrying is becoming less acceptable, also the transport of limestone is costly and contributes to environmental pollution. Because of the reduction in the total number of factories in operation, a process which affects many countries, the remaining factories increase their production rate and eventually are limited by their lime kiln capacity requiring a large capital investment.

There are several possible options to reduce lime usage, amongst which are:

- reduce lime to process whilst keeping essentially the same process (but suffer the consequences).
- reburn lime from process with a small make up of new lime.
- change the process radically to eliminate the use of lime.

This last option has been proposed and is being intensively developed by Amalgamated Research Inc. involving the use of their separation process based on ion exclusion resin technology (8). The process, if it can be established on a commercial scale, will offer total elimination of all lime kiln and processing operations as well as a very high sucrose recovery and hence very little molasses production. Another approach is the process developed from Ferrara University involving the direct crystallisation of raw juice to yield a raw sugar for refining and a molasses (19). As initially developed this process produces more molasses than the conventional lime based purification scheme because the juice impurities are not removed. Hence it may not be cost effective without further development. Such a development might be to combine raw juice crystallisation with molasses desugarisation, using ion exclusion resin techniques. Other processes might be based on total deionisation, as is used for chicory juice processing to yield a fructose product (7).

There are a range of processes which might offer an alternative to the use of lime but it is not yet clear that any will be universally as cost effective unless the cost of using lime increases.

Lime reburning has been carried out for many years in the Spreckels Sugar Company, now part of Imperial Holly, using a rotary kiln with a small make up of lime from another factory to allow a "blow down" of the process (9,11). This controls the build up of materials which inhibit the purification process, such as silicate. It is possible, perhaps even probable, that more companies will turn to this process to reduce their need for imported limestone. The lime reburning approach and the ion exclusion methods save the limestone but still have similar or greater fuel demands so will continue to add to the carbon dioxide load from fossil fuels.

In this paper we are concerned with the first approach, that of reducing lime usage further using conventional technology. If this could be achieved it would allow reduced limestone and fuel consumption which would give financial benefits in the interim period whilst the lime elimination processes are being developed. The consequences of reducing lime to process are discussed in several key areas and the opportunities to reduce lime use are considered.

In juice purification by liming and carbonation there are complex chemical processes in operation involving interactions between many juice components and calcium, and several degradation reactions involving high pH and temperature. In the discussion below, the various known purification reactions are considered in the light of results obtained by us and by other authors.

PRACTICAL STUDIES WITH SMALL PILOT PLANT PURIFICATION SYSTEMS AND IN THE LABORATORY

The majority of our experimental work has been carried out using a small pilot continuous juice purification apparatus, most often based at a factory site, to allow access to factory juice and of course allowing direct comparison with the results from the full scale process.

The pilot apparatus allows the simulation of a simple two stage carbonation with an intermediate 45 minute retention clarifier. Pure carbon dioxide gas is used for practical reasons but apart from this the apparatus simulates factory operations well at a flow rate of 300 cm³ per minute. First used in the early 70's the model

continues in operation today. More detail can be found in the earlier paper (15). The main advantage of this model is that it does match the chemistry and physical parameters of the factory well.

Some work has also been carried out using a batch process for the 2nd carbonation. The batch 2nd carbonation results are representative of what can be achieved in the continuous process. Operating back at the laboratory using stored juices is much less satisfactory mainly due to the increased invert levels experienced from deep frozen juice and hence most of the work has been done at a factory site during campaign.

RESULTS AND DISCUSSION

Elimination of anions as insoluble calcium salts

In a series of experiments where we operated with a range of lime levels over several days the following results were obtained:

Figure 1 shows the relationship between the measured 2nd carbonation lime-salts and the CaO percent on beet using a factory draft value of 115% (in practice the lime was proportioned to the juice flow and then the factory draft used to calculate percent on beet). Clearly the total lime-salts are dependent on the lime usage especially below about 1.5% on beet.

The lime-salts measurement is seen as an important parameter by factory staff but it is the consequence of such a complex series of effects, beginning with the beet composition and ending with the control parameters for second carbonation, that it cannot be used as a diagnostic test. In practice the lime-salts value depends on:

- the "natural alkalinity" of the beet, itself the balance, in the beet composition, of the acid forming components against the calcium precipitable anions.
- the amount of invert produced during extraction and the organic acids produced by bacterial activity before, during and after diffusion.
- additives such as, principally, sulphuric acid and calcium salts such as gypsum used in diffusion and as pressing aids.
- the elimination of anions during purification, principally in 1st carbonation.
- the elimination of calcium as the carbonate in 2nd carbonation.

In this paper we are only concerned with the elimination of anions during purification, making the assumption that all the other factors remain unchanged.

To understand this elimination in more detail, and its effect on the lime-salts value, it is necessary to consider the reaction between the calcium ion and the organic and inorganic anions present in juice.

McGinnis gave the major calcium precipitable juice anions as phosphate, citrate and oxalate with significant amounts of malate and sulphate (12). European juices have very similar components and the consequences are the same. Both phosphate and oxalate are almost completely eliminated by precipitation before and during 1st carbonation and we could find no correlation between the residual levels and the lime usage. While the residual phosphate is of little or no importance, the residual oxalate is important not because it affects the lime-salts value - it has no detectable effect on this value - but because it is the major scaling agent in beet factories. When we look wider within the factories we find thin juice oxalate values of from 0 - 20 mg/litre which indicates that the story around oxalate elimination is more complicated than it might at first seem. To enable a high elimination of oxalate it is necessary to achieve a high concentration of calcium ion in solution and to maintain this long enough for the supersaturation of calcium oxalate to fall. In the simultaneous defeco-carbonation system the greatest calcium ion concentration should be at the point where the milk of lime is added. However, the equilibrium concentration of calcium oxalate is not reached quickly and so the total residence time during carbonation may be a controlling factor in oxalate elimination.

The amount of lime used may be important since the calcium available in solution is restricted due to the poor solubility of calcium hydroxide in juice. Silin says that if 2% CaO is added to a normal raw juice only about 0.25% will be in solution with the remainder being in suspension (16). Of the soluble part only about 0.07% will be as free calcium ions, the remainder (0.18%) being associated with sucrose as a saccharate. If the phosphate and oxalate, which are capable of almost total precipitation with calcium, are considered, they are equivalent to about 0.07% CaO (subject to beet composition). Hence they can quickly remove all of the free calcium. Taking the citrate, malate and sulphate into account a sink for about 0.13% CaO is available. So the elimination of all of these compounds depends on these two equilibria; that between suspended and dissolved calcium hydroxide and that between calcium saccharate and free calcium.

We do not have an insight into the state of these equilibria during and before carbonation, but it is likely that they are the controlling stages in the precipitation of the calcium salts and therefore the ability to achieve high dissolved (and free) calcium ion will depend on the quantity (and surface area) of the CaO which is used in the process. In the case of calcium phosphate the solubility product is low and almost complete elimination is always achieved. For calcium oxalate the situation is similar but the small residual oxalate concentration has an enhanced importance due to its scaling potential later in the process.

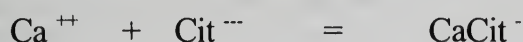
A question often asked is - could the residual oxalate come from other reactions in the process and not from the initial beet oxalate? Studies have shown that the residual oxalate is not formed as a consequence of invert degradation which removes one potential source from consideration (4). The hydrolysis of the amide of oxalic acid, oxamic acid, a minor juice component, will release oxalate but the reaction is relatively slow and should not be a significant source of residual oxalate (17). Although we could not demonstrate any relationship between residual oxalate and CaO to process, one should exist and oxalate levels could be expected to rise as lime is reduced.

When we look at the results found for sulphate elimination we can see (Figure 2) that the effect of reducing CaO to process follows the same pattern as for total lime-salts, that is, there is an increase in residual sulphate at low CaO addition.

Elimination of sulphate is always poor and we record values of from 30 to 50% elimination in our factories. However, the response of sulphate to CaO levels is not critical and elimination of more than 60% is difficult to achieve. If we were to reduce CaO to process to about 0.6% on beet we could expect to reduce average sulphate elimination to about 30%. The problem here is that we are big sulphate users both as sulphuric acid to control diffuser pH and as gypsum to increase pulp pressing performance. A penalty in terms of molasses production is going to be incurred as a result of reducing lime usage. The same effect is found for the elimination of malate.

Citrate is an anion which can lead to misunderstanding in the factory. Figure 3 shows the effect of reducing the lime to process on citrate elimination. Normally the elimination is high at around 90 - 95% giving residual levels of about 10 mg/100S or about 15 mg/litre. Again there is a strong dependence on the lime usage especially below 1% on beet so that if we were to operate at 0.6% lime on beet there would be about 100 mg of citrate per 100S, that is 10 times the value at 2% lime.

One feature of citrate is its association as a complex anion with calcium. This has been suggested to form according to the equation (5):



where the complex anion may account for more than 90% and probably never less than 50% of the calcium in solution. The remaining free calcium cation is available for precipitation as the less soluble oxalate salt later in the process when the calcium oxalate solubility product is exceeded.

In practice this effect would make the normal thin juice lime-salts titration fairly meaningless as an indicator of scaling potential at very low CaO to process levels. The EDTA titration measures the total calcium, both free and complexed whereas only the free cation can form salts. The proportion of free calcium depends on the residual citrate concentration after carbonation. Hence although the lime-salts value increases as CaO to process is reduced, the scaling potential does not increase in the same manner due to the increased citrate concentration.

In addition, the presence of citrate at representative levels was found to increase the solubility product of the sparingly soluble calcium sulphate by about 3 times. If this is also the case for oxalate, then there may be no greater scaling potential at 0.6% CaO to process than at 1.2% where we have shown that it is possible to use antiscalant compounds to cost effectively control deposition.

Degradation of organic compounds at high pH

Juice purification is carried out mainly at a pH equal to or above 11.2. At such a high pH the hydroxide concentration is sufficiently high to cause degradation of the invert sugars, the amides (glutamine and asparagine) and even of sucrose. It is necessary to degrade the amides and the invert sugars in order to have a purified juice which is reasonably thermally stable during evaporation so that the pH cannot fall to the extent that there is significant sucrose hydrolysis and colour production is minimised. However, the pH necessary to degrade these compounds can be achieved without high CaO concentrations.

Figure 4 illustrates the effect of increasing alkali addition to a typical raw juice. It can be seen that the target 1st carbonation end point of pH_{20} 11.2 (about pH_{85} 10.7) can be achieved with as little as 0.3% CaO on beet. This pH must be achieved in order to cause the most effective precipitation of protein materials from the juice.

Is this pH sufficient to carry out the degradation reactions or is a higher pH essential?

It could be argued from the short time at which higher pH is present in the defeco-carbonation system that a higher pH is not essential since the results obtained are already similar to those of a hot main liming system. However Figures 5 and 6 show the experimental results obtained in the degradation of glucose at high pH's in a solution containing only glucose and lime initially

In Figure 5 the reaction has been carried out in a concentration of lime equivalent to 1.2% CaO on beet (i.e. about 1.04% on the solution). This gives a pH of about 12.5 throughout the experiment. The glucose is destroyed very quickly so that after 10 minutes, more than 96% of the original glucose has been destroyed. After about one hour L-lactic acid, equal to 32% of the initial mass of the glucose present has been formed. In this reaction it is known that both D- and L-lactic acid are formed in equal amount so in total 64% of the glucose is converted to DL-lactic acid .

When the reaction is carried out at pH 11.2 (Figure 6) the degradation of the glucose is much slower but after 60 minutes 90% of the glucose has been eliminated, 17% being converted to L-lactic acid; that is, a total of 34% of the glucose mass is converted to DL-lactic acid, much less than at the higher pH.

Figures 5 and 6 also illustrate the other major difference in the response of this reaction to pH. About 2.4 times as much colour is formed at the lower pH when compared to the higher pH model. This is an important factor and is a major advantage of the main liming system over the simultaneous liming and gassing approach.

Experiments involving fructose, which is always the minor component of the invert sugars in factory juices show the same effects as for glucose but with faster degradation rates such that the whole of the fructose is eliminated in 60 minutes at pH 11.2. Again more colour is made at the lower pH.

When the degradation of glucose is studied in raw juice under mild carbonation conditions (Figure 7), it can be seen that the response is very similar to the model solution and again about 95% is destroyed in 60 minutes. Some of the L-lactic acid increase now originates from fructose .

It can be concluded that provided there is a total residence time of close to 60 minutes at a pH of 11.2 there should be no significant change in invert elimination as lime to process is reduced. This was shown to be the case in the continuous laboratory system with the results shown in Figure 8 where about 70% elimination was consistently achieved.

If we look at the consequence of exposing glutamine, the major amide in beet juice, to high pH conditions its degradation can easily be seen (Figure 9).

At 1.2% CaO on beet glutamine breakdown is rapid in the model solution, comprising 0.14 g/l glutamine, being about 60% complete after 10 minutes, a typical main liming retention. Some glutamic acid is formed but the

major end product is pyrrolidone carboxylic acid (not shown here). At pH 11.2, achieved with 0.25% CaO on juice, the reaction is much slower. After 55 minutes, the maximum time available at this pH in a simultaneous gassing system with clarifier, the reaction is about 40% complete.

In raw juice (Figure 10) the difference is less radical so that at 1.2% CaO on beet again about 59% is destroyed in 10 minutes whereas at pH 11.2 the average of two experiments showed 77% destruction in 55 minutes. Considering that a main limed juice goes on to receive perhaps 15 minutes at pH 11.2 it seems that there would be little difference, with respect to glutamine elimination, in the performance of a main liming system operating at 1.2% CaO on beet and a defeco-carbonation system operating at 0.25% CaO on beet.

The reaction rate of glutamine degradation has been studied in greater detail by Buczys, *et al.*, (2,3) who showed that over the range of conditions experienced in juice purification systems, the main controlling parameters were the juice pH and the temperature. Figure 11, simplified from Buczys (3), demonstrates the variation in the rate of reaction with pH at constant temperature (90°C). Below pH 10 the effect of pH is minimal whereas in moving from pH 11.2 to pH 12.5 the rate increases by 4 times. This explains the rapid amide degradation under main liming conditions. Buczys showed that the sucrose concentration also had a pronounced effect. Sucrose at high pH reacts as an anion and catalyses the degradation reaction which accounts for the difference between raw juice and the model solutions. The consequence of this understanding is that we should expect there to be no effect on amide degradation rate of reducing CaO to process over a wide range in a defeco-carbonation process since the process pH will be constant. It was possible to confirm this by operation of the pilot plant system giving the results shown in Figure 12. With a constant retention time and temperature together with little variation in raw juice sucrose the elimination of glutamine expressed as a percentage of the raw juice content was about 53% over the range 0.35 to 3.05% CaO on beet. To achieve a better elimination would require either a longer retention time in the 1st carbonation and clarifier or a higher process temperature.

Sucrose is also degraded at high pH but in this case we are trying to minimise the loss rather than increase the degradation. The factors which help in the elimination of the invert sugars and the amides will adversely affect the sucrose loss. Hence we need to minimise temperatures and residence times and operate as close to pH 8, the pH at which inversion is minimised, as possible. Work by Bichsel showed that losses during purification were probably around 0.1% sucrose when extrapolated to our conditions of pH and retention time (1). More recent work by Eggleston (6) has looked at a wider range of conditions and should give more definitive results together with a possible means of establishing actual factory losses by looking for the end products of sucrose degradation.

Absorption effects

One of the important properties of the fresh calcium carbonate precipitate is its ability to absorb components, especially colour materials, from solution. This effect is responsible for much of the colour elimination during carbonation and should be a function of the surface area of the calcium carbonate crystals exposed to the solution. Reducing the quantity of lime to process should therefore have a direct effect on the colour of the purified juice.

Figure 13 shows the way in which 2nd carbonation juice colour responds to the reduction in lime to process. As lime usage is reduced the juice colour increases. Below about 1% CaO on beet the increase in juice colour is

such that the juice colour at 0.6% CaO is 40% greater than that at 1.2% CaO to process and nearly 90% greater than at 2% lime addition. These colour increases will be reflected in the thin and thick juice colours and would lead to a large increase in white sugar colour if no other colour reduction measures were taken.

Physical parameters

Experimental work around the chemistry of purification indicates that with some disadvantages it would be possible to operate a conventional defeco-carbonation purification at much lower lime usage than we have currently achieved. In practise the major difficulties would arise in the separation of the precipitated calcium carbonate mud from the juice. Two separation processes are involved in British Sugar. All factories have a clarifier system and also a rotary vacuum filtration system, to achieve this separation. Therefore we must be concerned about both the sedimentation rate of the mud and its filtration rate.

We have established that simply reducing the lime to process causes changes in the sedimentation and filtration rates of 1st carbonation juice. Table 1 illustrates these effects, measured on the continuous apparatus at one factory. In these experiments the raw juice from the factory already contains returned clarifier underflow (Wilfley mud). The value shown as % CaO on beet is the added milk of lime used in the pilot plant. Sedimentation rate is calculated from the time needed for the break to pass between two points, 10 cm apart, in a 250 cm³ measuring cylinder. The filtration time is the time taken to filter 100 cm³ of unclarified juice through a 47 mm diameter 8µm pore size membrane at a vacuum of 15 inches of mercury.

The sedimentation rate is almost independent of the amount of lime used. Any reduction in settling rate could always be recovered by the addition of a cationic flocculant which would be expected to increase the rate by up to 2.5 times when used at about 1 ppm on juice. The filtration time increases with reduced lime and at 75 seconds would be about 50% above the limit for current filtration capability.

When it was clear that filtration rates could be a major issue in any further development we considered whether there were any options which might improve this parameter. A patented process has used a regenerated lime mud to enable factories to reduce their lime consumption from traditionally high usages to around 1.2% and lower in some cases (13,14,18). In this process the lime mud is cleaned by first reducing its pH to about 8.5 using carbon dioxide gas. This causes the release of much of the absorbed components which can then be separated together with the finest particles of calcium carbonate. The cleaned lime is then regenerated by treatment with fresh calcium oxide before use as a substitute for some of the milk of lime used in processing. Part of the concept is that the regenerated lime is able to absorb more impurities on its active surface and part is that it operates as a filter aid.

A series of experiments was carried out to discover whether using a regenerated mud would allow operation at very low lime levels with the results shown in Table 2.

In the first experiments (a) 1.2% CaO on beet was used as the base case reproducing the factory situation. The effect of returning clarifier underflow (Wilfley mud) to raw juice is to greatly increase the settling rate and reduce the filtration time. This demonstrates why return of underflow has been a necessary element of our factory lime reduction programme.

In the second set of experiments (b) the milk of lime to process has been reduced to 0.6% CaO on beet. Both the settling and filtration rates are worse than the base case. Again recycle of Wilfley mud greatly improves the settling rate and also improves the filtration time although it remains very poor. When regenerated lime mud is used it has no beneficial effect at all, at this low milk of lime level. The settling rate and the filtration time are returned to their values at 0.6% CaO alone. When both Wilfley mud and regenerated mud are added simultaneously the end result is only marginally better than for the regenerated mud alone.

In the final experiments (c) where much more regenerated mud is added (equivalent to 1.2% CaO on beet) there is no benefit for settling and filtration becomes impossible.

SUMMARY

Working with the pilot purification system indicates that it may well be possible, in practice, to operate a defeco-carbonation with UK beet at lower than 1% CaO on beet. The best option appears to be to establish a retention time in the 1st carbonation and clarifier sufficient to achieve the elimination of invert and glutamine equivalent to the current performance at 1.2% CaO on beet and to recycle clarifier underflow sufficient to achieve acceptable settling and filtration rates. The addition of flocculants to facilitate settling might be necessary and a significant increase in RVF area would be needed. Table 3 shows the changes we would expect by operating at much lower lime addition rates compared to current performance at 1.2% CaO to process.

A next step might be to consider operation at about 0.8% CaO on beet, which in our experience is consistently possible on the model system. Lime-salts would undoubtedly increase but could be contained to some extent by addition of sodium or magnesium after 2nd carbonation. The purity reduction as shown is calculated by assuming that the increased lime-salts are as a result of sulphate, citrate and malate anions in their normal ratios. Most of this purity loss is unavoidable, as only the cations can be easily reduced to less melassigenic species, e.g. by exchange for magnesium. Juice thermostability would be very similar to present, as both invert and glutamine are largely unaffected by lime usage.

The major uncertainties would be the scaling behaviour of the juice with higher lime-salts and the ability to produce current white sugar colours with the increased juice colours. Faced with these uncertainties and the cost of decreasing juice purity it could well be argued that current lime usage is optimum at around 1.2% on beet. However, this was also the argument when lime consumption was more than 2% on beet.

CONCLUSIONS

Working with current UK beet quality it is possible to operate the defeco-carbonation process, involving simultaneous liming and gassing with juice clarification, at 1.2% on beet without adverse consequences. Experiences in Europe indicate that the same is possible with classical systems of processing involving pre-liming and main liming.

Operation of a small pilot scale system has shown that it may be feasible to operate the carbonation system at even lower lime levels but with some significant adverse consequences.

The major cost disadvantage at very low lime levels is the increase in lime-salts with their effect in reducing thick juice purity and therefore reducing molasses exhaustion.

The increase in lime-salts is mainly due to reduced elimination of citrate, sulphate and malate anions. The increase in residual citrate would reduce the value of the lime-salts titration as an indicator of scaling potential of a thin juice.

The major operational disadvantage at very low lime levels is the poor filterability of the 1st carbonation juice and of the settled mud. To operate at 0.6% CaO to process, which can be achieved in the pilot system, would require an increase in filter area (RVF) estimated to be at least two times, and perhaps as high as four times, that used at present.

Settling in the clarifier is not considered to be a major issue and experience shows that cost effective flocculants can be used to increase settling rate by at least two times allowing some margin of safety.

Attempts to improve the filtration and settling of 1st carbonation juice by recycling RVF mud had mixed results. The best effects were seen with untreated mud which was found to have dramatic beneficial effects on both settling rate and filterability of 1st carbonation juice. Compared to these effects all attempts to operate at significantly less than 1% CaO to process with some form of treated, that is cleaned, recycled mud were unsuccessful.

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Table 1. Effect of reduced lime addition on first carbonatation settling and filtration rate.

Milk of lime addition (%CaO)	Settling rate (cm/min)	Filtration time (s)
1.2	25	36
1.0	27	43
0.8	18	49
0.6	21	75

Table 2. Effect of recycling clarifier underflow.

	Milk of lime (%CaO)	Wilfley slurry (%CaO)	Regenerated mud (%CaO)	Settling rate (cm/min)	Filtration time (s)
a.	1.2	0	0	7.9	122
	1.2	0.6	0	32	22
b.	0.6	0	0	4.8	272
	0.6	0.6	0	27	150
	0.6	0	0.6	4.6	300
	0.6	0.6	0.6	5.3	185
c.	0.6	0	1.2	3.9	>300

Table 3. Expected juice purification at reduced lime to process.

%CaO to process	2.0	1.2	0.8	0.6	0.25
Lime-salts (CaO/100bx)	0.031	0.055	0.086	0.126	>2
Purity change (%)	0	-0.065	-0.15	-0.26	-0.45
Invert elimination (%)	70	70	70	70	70
Glutamine elimination (%)	50-70	50-70	50-70	50-70	50-70
Relative colour increase	0	1.3	1.6	1.8	2.3

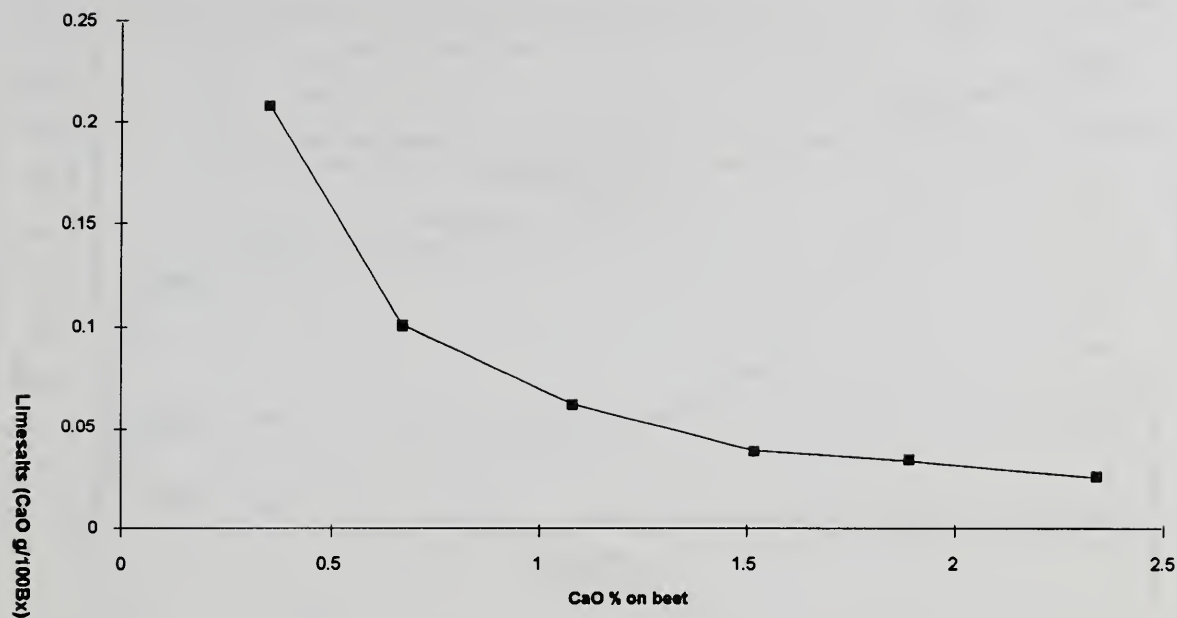


Figure 1. Variation in 2nd carbonatation lime-salts with lime to process.

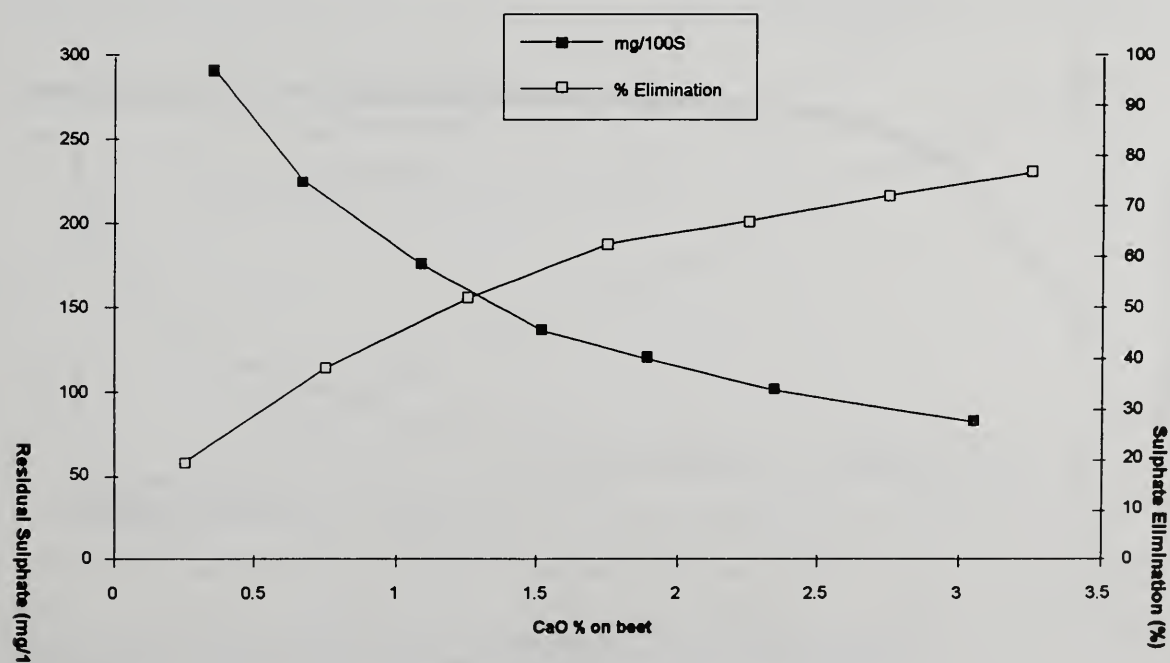


Figure 2. Variation in sulphate elimination with lime to process.

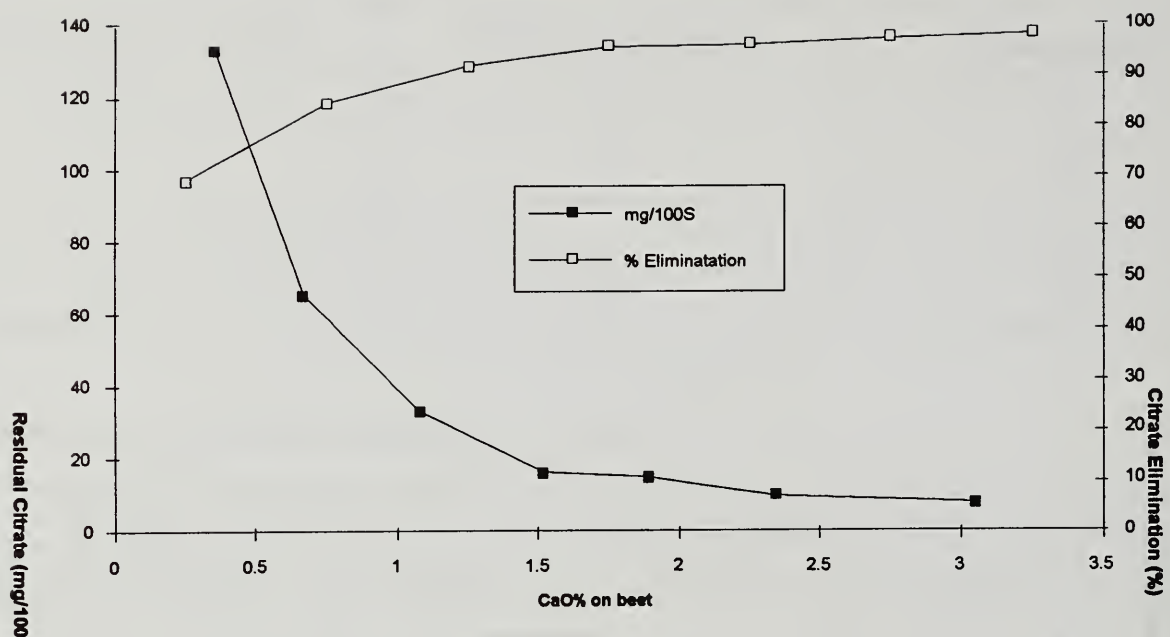


Figure 3. Variation in citrate elimination with lime to process.

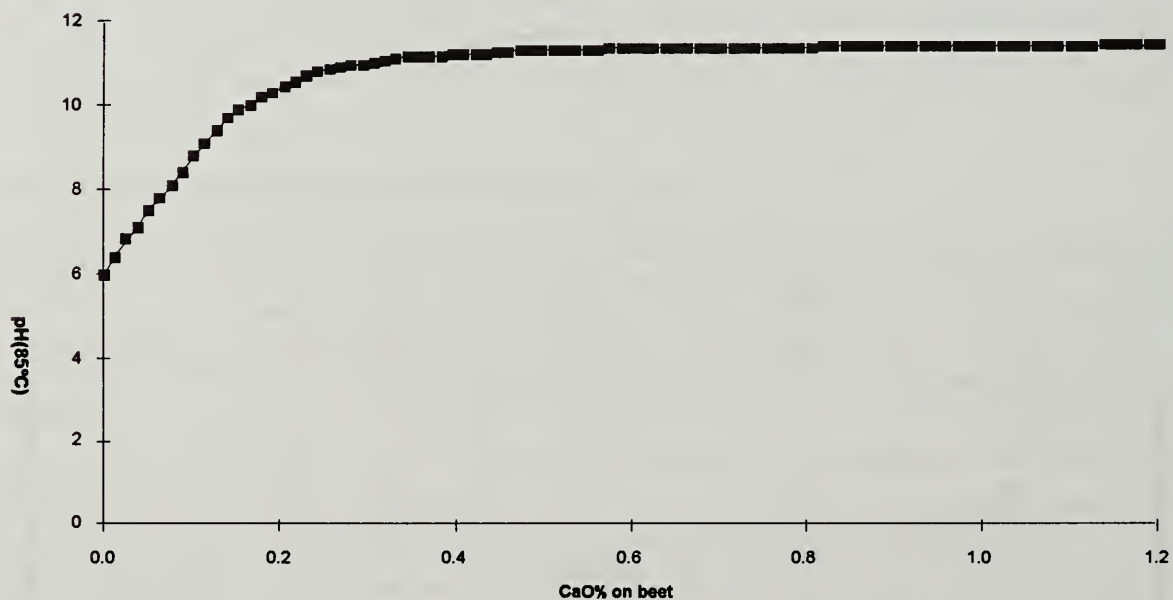


Figure 4. Effect on raw juice pH of addition of calcium hydroxide.

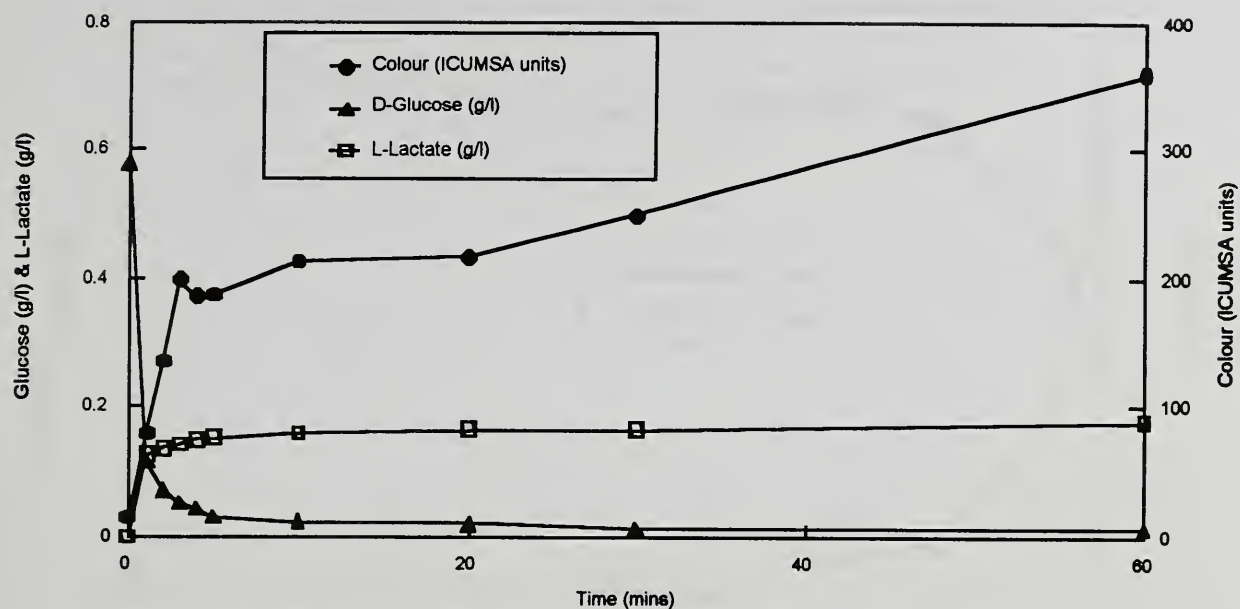


Figure 5. Degradation of glucose at main liming pH in model solutions.

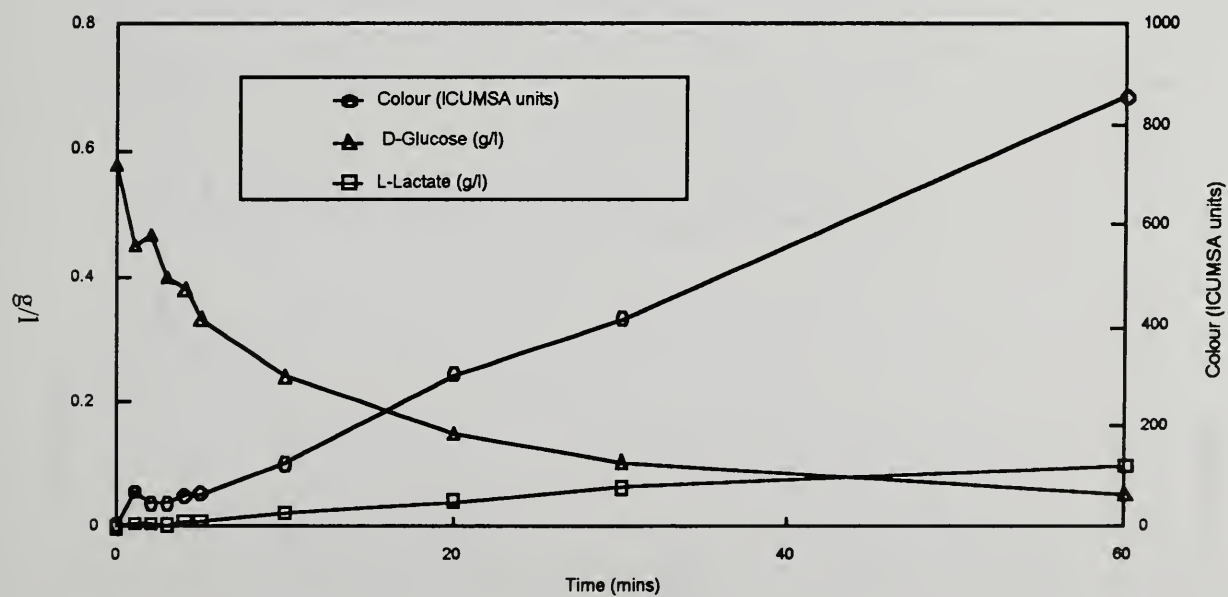


Figure 6. Degradation of glucose at mild carbonatation pH in model solutions.

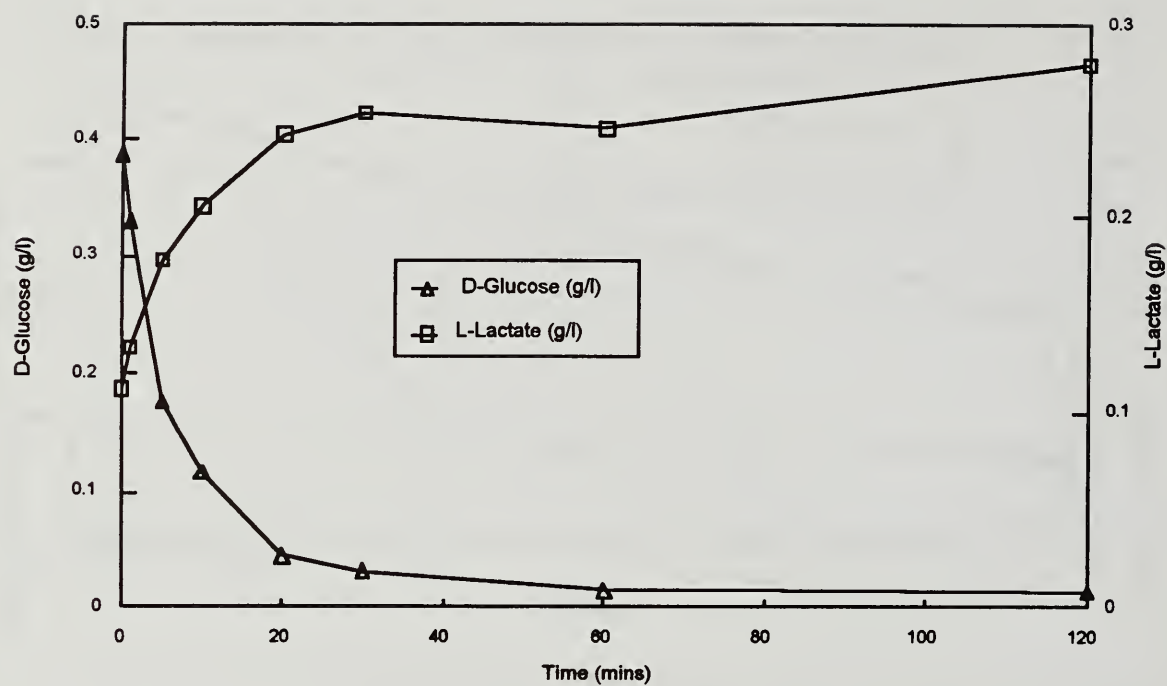


Figure 7. Degradation of glucose in raw juice at pH 1.2.

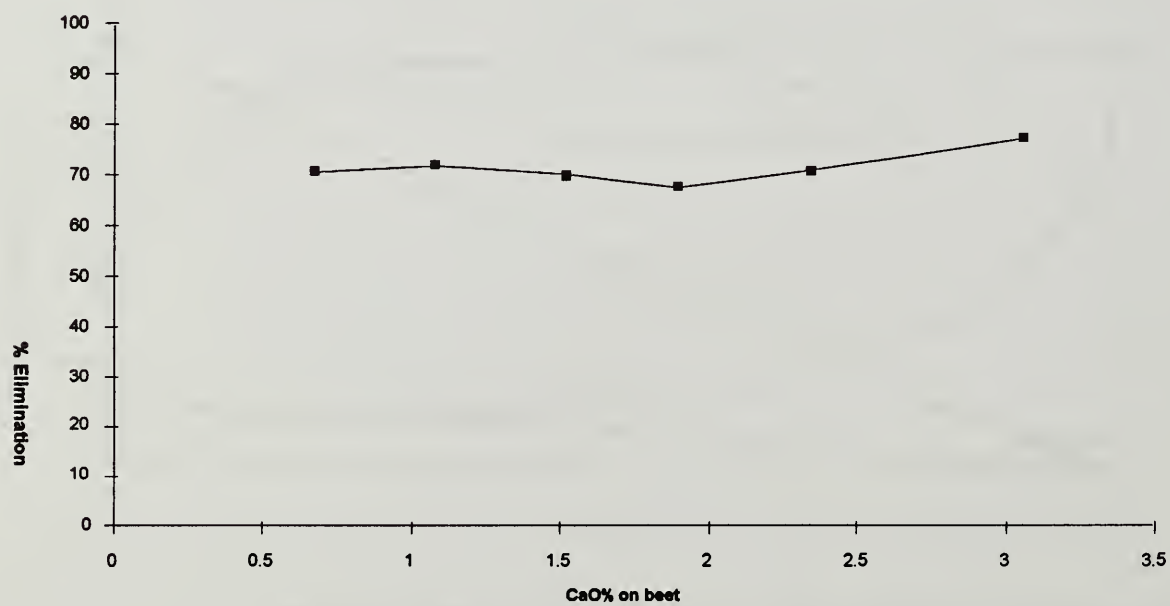


Figure 8. Variation in invert sugars elimination with lime to process.

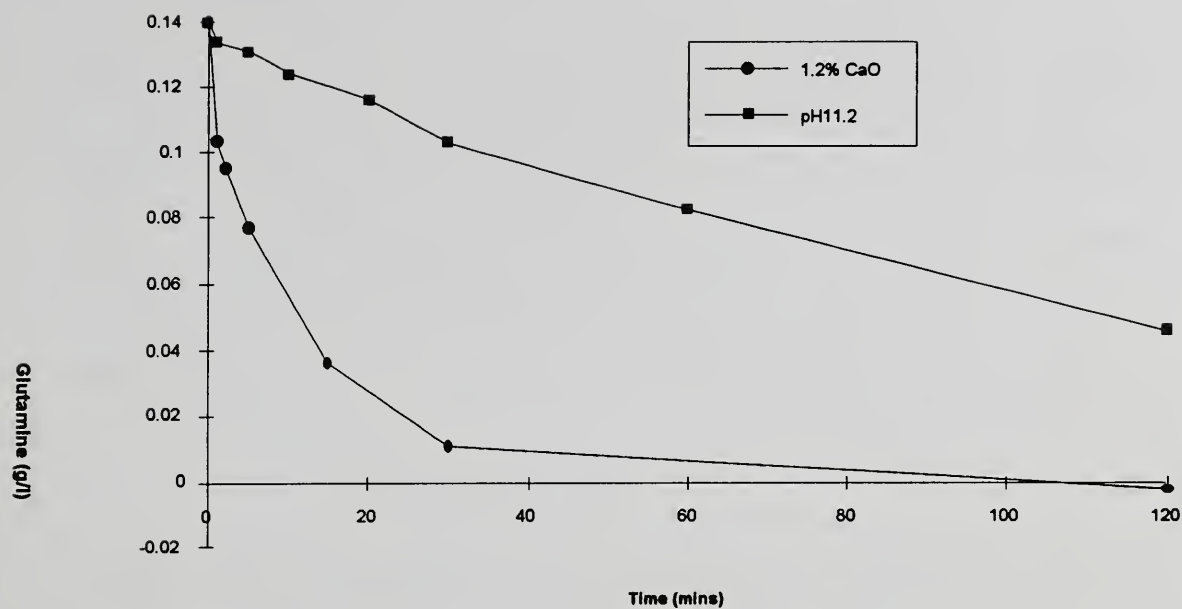


Figure 9. Degradation of glutamine in model solutions.

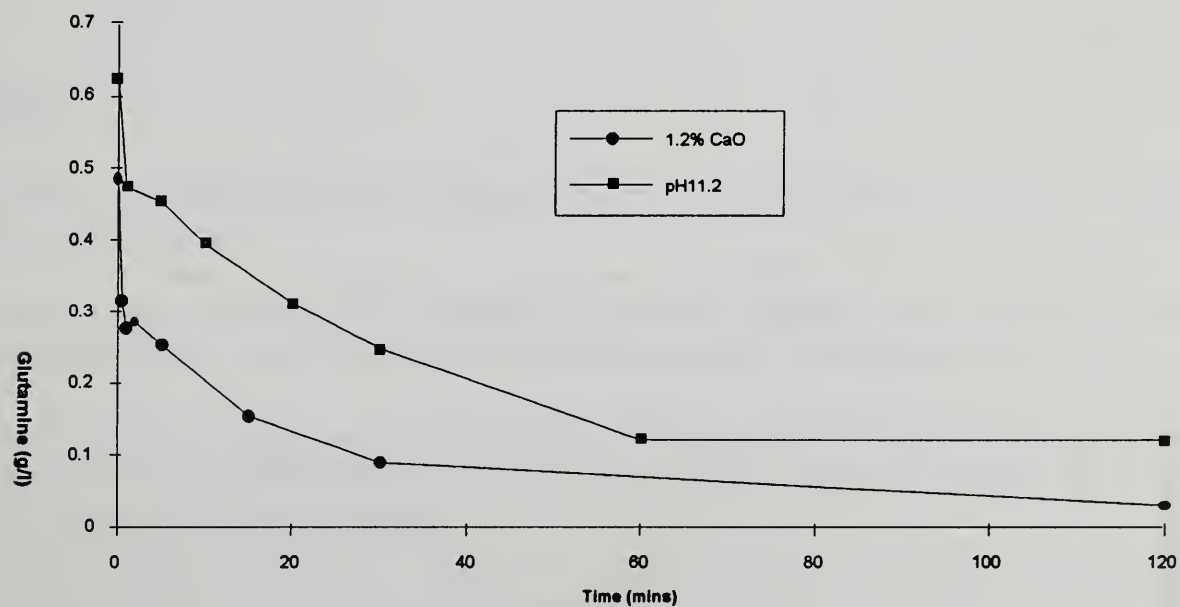


Figure 10. Degradation of glutamine in raw juice.

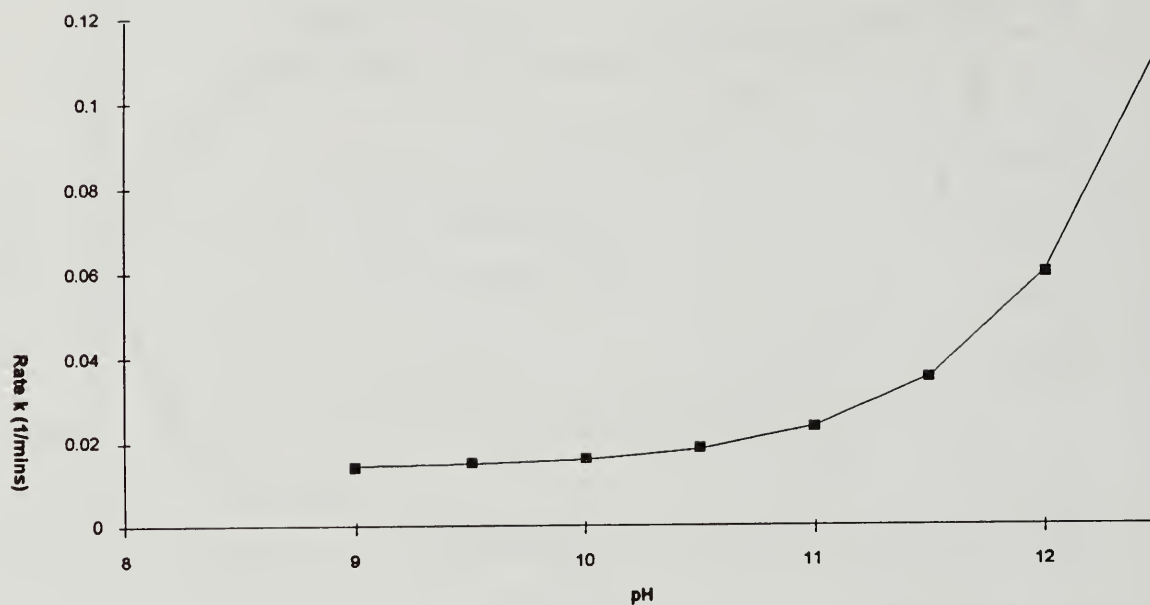


Figure 11. Degradation of glutamine in purification as a function of pH.

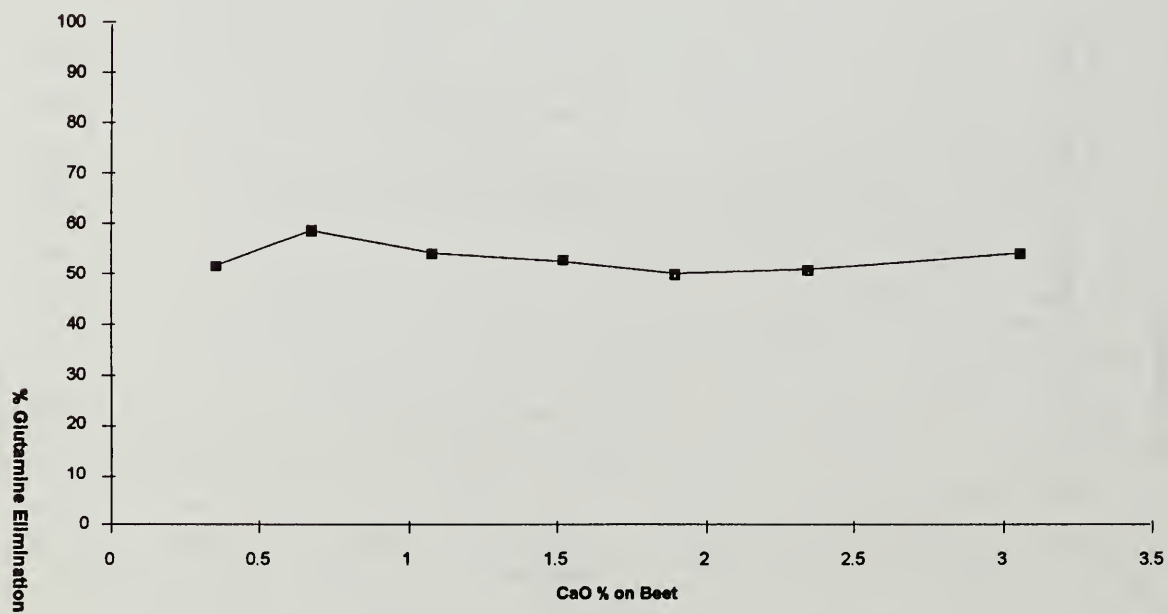


Figure 12. Variation of glutamine elimination with lime to process.

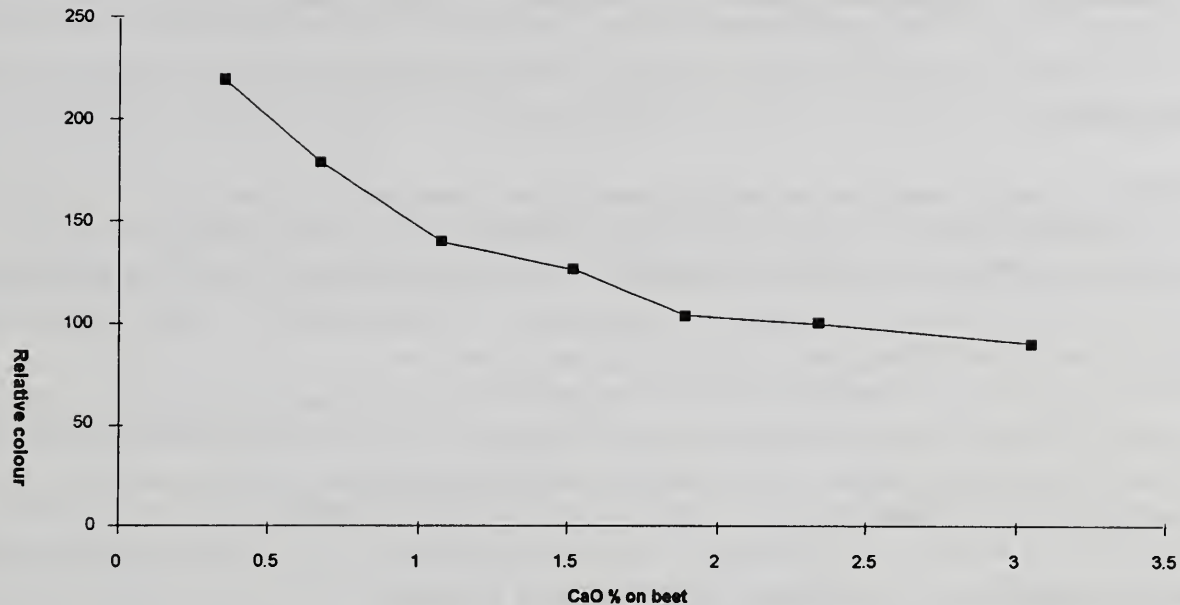


Figure 13. Variation in relative colour elimination with lime to process.

DISCUSSION

Question: Have you noticed the need for additional lime toward the end of the campaign when beets deteriorate and raw juice purities drop?

Sargent: We try to maintain the same lime usage throughout the campaign. Towards the end of February there will be significantly more invert from the beet, and factories may use a little more lime.

At the beginning of the campaign many of the factories can be running at 1% CaO on beet and as you saw in the slides the company average for the whole campaign is around 1.2%. I do not believe that any factory has gone over 1.4% even at the end of the campaign.

FUTURE OF MEMBRANE TECHNOLOGY IN THE SUGAR INDUSTRY: IMPORTANCE OF PROPER TESTING

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INTRODUCTION

Within the last few decades membrane technology has become a standard unit operation in the water treatment, dairy and food industries (1). Although the first efforts to study membrane applications in the sugar industry were made in the late 1960's (2, 3), one can count just a few installations both in beet and cane factories. For years most of the researchers were focused on replacement of conventional juice purification by membrane filtration (4,5). Due to the differences in strategical goals of various companies it is still difficult to discern whether there is a place for economically feasible "straightforward" membrane applications. Over the last few years the research activity has been focused on the development of new technologies incorporating membrane processes as one of the unit operations. The current and potential applications of membrane filtration in beet and cane sugar industries are reviewed below.

The following terminology and abbreviations will be used in this paper.

Permeate	The portion of a feedstock that passes through a membrane.
Permeate flux	Specific flow rate of permeate, usually expressed in LMH (liters/(m ² hr)) or GFD (gallons/(ft ² day)).
Concentrate (or retentate)	The portion of feed rejected by a membrane.
Transmembrane pressure (TMP)	TMP is calculated as the difference between the average pressure on the feed side of the membrane and the permeate side pressure. Some companies use an abbreviation ATP (average transmembrane pressure).
Concentration factor	Feed-to-concentrate volume ratio.

BEET SUGAR INDUSTRY

1. A process for beet juice chromatographic separation has been developed and patented by Amalgamated Research Inc. (6). Membrane filtration is considered an efficient form of juice pretreatment. Filtered raw juice is softened and evaporated; the resulting syrup is purified using chromatographic separation. The process has been successfully piloted for five years. Details about the process can be found in a paper by M. Kearney and D. E. Rearick (7).
2. Raw juice micro/ultrafiltration can be integrated into existing sugar plants. Permeate is more likely to be post-treated with lime to achieve a purity rise comparable to conventional purification. However, filtered

raw juice can be stored and processed later. Bacteria counts in the concentrated micro filtered juice are several orders of magnitude lower than in thick juice sent to storage. After appropriate testing the process may be useful for factories with lime kilns undersized to handle an increased slice rate.

3. Reverse osmosis (RO) with polymeric membranes was successfully applied for pre-concentration of an effluent stream from molasses desugarization plant in Southern Minnesota Sugar Cooperative (8). It appears to be economically feasible to use RO for dilute solutions and follow it with conventional evaporation as concentration exceeds 12-15% DS.
4. Press water is a very dilute stream containing about 3-4% of total sugar entering the factory. It is normally recycled back to a diffusion stage after pulp presses. Membrane filtration can be applied for both press water sterilization and suspended solids removal.

CANE SUGAR INDUSTRY

1. Membrane filtration is an efficient process for clarification of mixed or clarified juice prior to softening and chromatographic separation (9). It has been proven that chromatography was capable of effective removal of cane non-sugars and color (10). With the new patent pending Coupled Loop Chromatography method (11) a much higher degree of juice purification can be achieved. The possibility of producing white sugar directly from extract is currently being tested. If product sugar quality satisfies the refined sugar standards, the process will also significantly increase the overall extraction in the cane mills.
2. Although the idea to use ultrafiltration for clarified juice was studied since the early 80's, a large-scale system was installed in Puunene, HI only several years ago. According to this process, clarified juice is ultrafiltered, softened and evaporated. The process produces VLC sugar. However, the product quality is not high enough to meet refined sugar standards. The membrane system utilizes Kerasep ceramic ultrafiltration membranes. Details on system parameters and operation can be found in the literature (12, 13).
3. The A.B.C. process involves membrane filtration of mixed cane juice followed by adsorptive decolorization (14, 15). Hollow fiber membranes made from a hydrophilic polymer are used for filtration of carefully prescreened mixed juice. The quality of sugar produced with the A.B.C. process closely approaches the requirements for refined sugar. Since mixed juice pH is most likely adjusted prior to filtration to reduce sugar losses using melassigenic chemicals, the overall extraction should be evaluated. Since neither ultrafiltration nor decolorization are capable of removing invert or ash from the juice stream, the concentration of these components in the final product stream is still a concern.
4. A similar approach to production of white sugar was proposed by M. Saska (16) where clarified juice was ultrafiltered and then decolorized with nanofiltration (NF) membranes. It was also suggested to use NF for reduction of color and viscosity in the mills and refinery streams (17).
5. Cane molasses membrane pretreatment prior to chromatography should be developed on a case by case basis due to tremendous differences in molasses properties from various sources.

6. MF/UF treatment of affination syrup in sugar refineries reportedly removes some polysaccharides and colorants and leads to improved processing characteristics and quality of final product (18).

New ideas on membrane applications continue to appear as new information on membrane performance becomes available. Priorities are significantly affected by new EPA regulations, size of a sugar plant, cost of power and membrane systems. Market changes may significantly affect the feasibility of membrane technology in the cane sugar industry. Most of the promising membrane applications are associated with new technologies. Additional purification methods following membrane filtration should be developed and integrated into existing plants.

REVIEW OF MEMBRANE PERFORMANCE

Although membrane separation characteristics strongly depend on the average pore size, the mechanism of separation is much more complicated than simple "screening". Formation of a dynamic layer, concentration of dissolved and suspended solids and presence of surfactants are among the factors critical for membrane performance. According to traditional classification, membranes are rated by pore size in the microfiltration range and by "molecular weight cut-off" (MWCO) in ultrafiltration. An approximate classification of membrane processes is shown in Table 1.

Analysis of the composition of various sugar juices and syrups can be helpful in evaluating expected membrane performance, although only experimental results can provide the true information on membrane performance. However, preliminary analysis may save time and resources for a test program and provide information on the nature of potential foulants.

Table 2 contains analytical data for a sample of beet diffusion juice from a factory in Southern Idaho, USA. A simple comparison of molecular size of juice constituents with pore size of membranes shows that most of the high molecular weight components (molecular weight exceeding several hundred thousand units) can be removed by microfiltration or "loose" ultrafiltration membranes. Because of the larger porosity, higher permeate flux is expected in this range of pore sizes. Using membranes with smaller pore size causes significant flux reduction without noticeable increase in separation.

Based on preliminary analysis of juice properties it may be concluded that membranes may be feasible for the removal of suspended solids, colloidal material and other high molecular weight compounds, but they perform poorly for separation of most of the dissolved non-sugar components.

Conventional purification methods in beet factories typically eliminate about 30% of non-sugars, which translates into 3.0-3.5 points purity increase. Analysis of data in Tables 3 and 4 can give an answer to the long-debated question whether membrane filtration can achieve the degree of purification comparable with conventional methods. Table 3 contains calculated numbers of purity increase corresponding to different levels of non-sugar removal for an 88 purity juice sample. Under the assumption that one half of the unaccounted non-sugars in Table 2 is rejected by ultrafiltration membranes, maximal theoretical non-sugar elimination cannot exceed approximately 10%. The purity difference between the feed juice and permeate increases with decrease in feed juice purity at the same degree of non-sugar elimination. Expected values of purity rise across a

membrane are listed in Table 4 assuming 10% non-sugar elimination. Therefore, expected purity change across MF or UF membrane should not exceed more than one purity point (may be slightly higher for low purity juices). This conclusion is confirmed by the experimental data obtained by various researchers. Most of the data in Table 5 correspond well with the theoretical analysis.

It is also important to analyze the influence of membrane filtration on other parameters critical for various processing steps. Color of sugar syrups is definitely one of the most important factors affecting the sugar end operation and properties of product sugar. A number of studies have shown that depending on molecular weight, colorants in sugar juices have different effects on product quality. Therefore, changes in color across the membrane affect crystallization both directly by reducing color of the solution and indirectly by eliminating high molecular weight colorants. The latter appears to have a significant effect on sugar quality, especially in cane applications. Table 5 contains some information on color removal from various technical sugar solutions. Although some of the data show influence of membrane size on efficiency of color removal, separate evaluation should be done for each application at different stages of processing.

Removal of dextrans is critical in both cane and beet sugar manufacturing. According to many sources (22) dextrans not only change the purity of sucrose crystals but also affect crystal morphology. The long chain dextrans present in sugar juices which are believed to cause problems during the crystallization step typically have molecular weight about 2 to 20 million Da. These dextrans are definitely rejected by both MF and UF membranes. Various researchers report different levels of dextran elimination across the membranes. The discrepancies obviously depend on the analytical method used. Typically, the haze method accounting for high molecular weight dextrans shows higher values of dextran elimination. A method developed by E. J. Roberts covering a wider range of molecular weights appears to be more accurate and indicates that low molecular weight dextrans are not rejected by the MF and UF membranes.

From the previous discussion it is clear that membranes with pore sizes less than 0.2 micron completely remove suspended solids and colloidal particles as well as microorganisms and spores. This fact has been confirmed in many studies by measuring turbidity, bacteria counts and suspended solids in permeate. In our studies we have also observed 10-20% reduction in hardness of beet juice, cane molasses and press water. Hardness reduction is most likely caused by precipitation of insoluble salts of organic or inorganic acids.

TEST PROGRAM DEVELOPMENT

Integration of new membrane technology into an existing plant requires extensive testing. A poorly designed test program may not only waste time and resources but also compromise a new, promising technology. It has been proven by many researchers that most membranes provide permeate of adequate quality. Therefore, the main goal of membrane process development should be selection of the most reliable and economical membrane. Because juice properties vary significantly during the course of campaign in both beet and cane sugar plants it is extremely important to observe long-term behavior and monitor changes in membrane characteristics. The following issues should be addressed in a well-designed pilot test program.

- Stable permeate flux
- Optimal recirculation rate
- Dependence of permeate flux (or membrane permeability) on concentration factor

S.P.R.I.

- Feed pretreatment requirements
- Membrane integrity and mode of failure
- Cleaning procedure
- User friendly control strategy
- Concentrate handling

Some of these factors are necessary for design of the membrane system itself, the others are critical for smooth transition of a new technology from pilot scale to commercial use. Since permeate flux depends on degree of concentration of feed solution, it is important to obtain reliable data within a wide range of concentration factors. For example, a typical flux vs. concentration factor curve is shown in Figure 1. Each data point in this curve should represent at least one full operation cycle. Reproducibility of the data points depends on juice quality, membrane conditions, harvesting, storage conditions and other factors. Therefore, duplication of data is critical for system design. The curve provides information for calculation of average flux per stage and required overall surface area. Ideally, an infinite number of stages in series gives maximum overall performance. In practical applications the required number of stages depends on system size, pumping requirements, number of modules in a stage, etc., and is usually limited to three to ten stages.

CAPITAL AND OPERATING COST

Only membranes with high porosity may provide enough throughput to feasibly treat the large streams of sugar juice. Therefore, most of the information discussed below is related to microfiltration and ultrafiltration processes. However, general analysis can be equally applied to nanofiltration and reverse osmosis.

Most of the applications in the sugar industry would impose the following requirements on membrane characteristics.

1. High permeate flux (a typical beet or cane sugar plant produces 1000-4000 gpm of juice at 14-18% RDS).
2. Temperatures above 85°C to prevent bacterial growth.
3. Presence of certain quantities of abrasive materials in the raw juice streams.
4. High concentration factors required to minimize sugar losses.

Selection of a membrane that meets all the requirements listed above presents a serious problem and a developmental challenge. Not only should the physical characteristics of a membrane meet the specifications, but the membrane should be able to maintain these characteristics for an extended period of time.

Since the cost of membrane replacement may be 20-50% of initial capital investment, evaluation of a membrane's service life is extremely important. Based on currently available information, membrane service life should be at least one to two campaigns for polymeric and four to five campaigns for non-polymeric (e.g., ceramic or stainless steel) membranes. The manufacturer's warranty may cover membrane replacement cost (usually on a prorated basis) but does not cover the expenses related to reduced capacity or factory shutdown. Therefore, early involvement of an end user in the process development is essential to reach the comfort level required to successfully implement this new technology.

The feasibility study of a new membrane process includes obtaining reliable estimates of capital and operating expenses. The selection of membrane type based only on cost per unit of surface area may lead to serious mistakes. A budgetary quote for a membrane system designed for a certain plant capacity and based on reliable pilot experiments can give the one and only estimate of capital cost. For example, low cost membranes providing low permeate flux may be more expensive overall than a competitive higher cost product. Similar mistakes can be made when energy use is determined based only on crossflow velocity.

In membrane filtration most of the energy is consumed by recirculation pumps that provide high fluid velocity creating shear on the membrane surface. Different membrane configurations require different crossflow velocities. For example, spiral wound elements with high surface to volume ratio typically use less energy per unit of surface area. However, this does not mean that they provide the most energy-efficient solution. If permeate flux is low, the overall energy use may still be significant.

Besides energy required for feed and recirculation pumps, operating expenses for any membrane system include the cost of membrane replacement and cleaning agents. Although the expenses related to cleaning chemicals usually represent a small portion of the overall operating cost, it is critical to evaluate the methods of their utilization or disposal.

COMMENTS ON PLANT AUTOMATION

Pilot Plants

A variety of membrane pilot plants are used in the industry ranging from simple manually operated units to highly automated installations. The level of plant automation should be determined by a membrane manufacturer or an end user depending on the project goals, e.g., feasibility study, data collection for a full-scale design, preparatory purposes, etc. Before starting a pilot test one should make sure that the level of automation is adequate for the purposes of the experiment. Insufficient automation of a pilot plant may result in poor quality of collected data and may compromise the purpose of an experiment or, even worse, idealize the process.

Usually minimal automation is required for short-term batch experiments. However, even in this case certain critical parameters, such as process temperature, should be controlled automatically. If a pilot plant is used for subsequent design of a full-scale plant, it is wise to provide as many control points as practical. Furthermore, the pilot plant should be capable of operating reliably for long periods of time. Maintaining reliable long-term operation of a pilot plant is a serious challenge without proper automation.

To illustrate pilot plant automation, a one stage ultrafiltration plant operating in a "feed-and-bleed" mode is shown in Figure 2. Enough control points are indicated to provide full automation. Operator involvement would still be required for sample collection or initiation of a cleaning procedure. Most pilot plants in the membrane industry are less automated than this simple example. The sample control strategy allows the permeate flow to be held at a constant level. The strategy allows automatic control of the recirculation flow, liquid temperature and pressures. Continuous opening of the permeate control valve will provide a gradual increase of TMP as the membrane fouls. Such a control strategy may be applicable for operation of hollow fiber

or ceramic membranes where a certain back pressure is allowed. The permeate-to-concentrate flow ratio is maintained constant to guarantee that the concentration factor does not change during the test.

The diagram in Figure 3 represents an alternative strategy where the transmembrane pressure (TMP) can be varied by opening or closing the flow control valve in the feed line. A similar result can be achieved by using a variable frequency drive (VFD) on the motor of a feed pump instead of a flow control valve. This strategy is preferable in cases where the back pressure on the permeate side may cause damage to the membrane. A good example is the operation of a plant with spiral wound elements where back pressure results in membrane delamination or breakage.

The data transfer from pilot plant to a full-scale installation will depend greatly on the quality of collected information. Table 6 provides some guidelines for pilot plant control strategy design. Other parameters critical for a specific feed stock such as pH, refractometric index, etc., should be included in the control scheme.

Industrial Plants

Automation of an industrial installation depends strongly on the local plant control philosophy. Most end users request instrumentation similar to what is accepted as standard for their plant. The automation package of a membrane system is usually integrated into the existing factory control system. Because of the different goals pursued by the operation of pilot and industrial plants the control strategy and number of control points may vary. The pilot plant should generally be more sophisticated than the industrial process in order to guarantee accurate and reliable scale-up data. This will also help in determining which parameters are most critical to control a full-scale installation. Typically the scale-up of an automation package from pilot plant to industrial scale should be accompanied by simplification. For example, the recirculation loop flow meter and flow control valves may not be necessary in an industrial plant if proper optimization was performed on a smaller scale. Most industrial installations do not control the concentration factor in each consecutive loop but rather let the system equilibrate internally. On the other hand, cleaning procedures are usually automated for industrial plants, whereas most pilot units are cleaned manually. An effort to save money by removing key components of automation may result in serious problems with plant operation and troubleshooting.

The configuration of an industrial system plays a significant role in determining the placement of primary sensors and defining the control strategy. The number of measuring instruments and control valves required for an industrial membrane plant is dependent on the number of modules in series or in parallel, frequency of cleaning and other considerations. In an example layout shown in Figure 4a one parallel line is reserved completely for cleaning. Each block in the diagrams represents a set of modules arranged in parallel using one recirculation pump. Although this type of configuration provides reliable operation it utilizes thirty percent more membrane area. The alternative scheme shown in Figure 4b illustrates a more "frugal" approach to system design which will, however, require more control valves to achieve proper module sequencing. Equipment manufacturers use different approaches to system design. End user involvement is important to make correct decisions at this stage of process development.

LONG-TERM TESTING

Constantly changing properties of sugar juices and syrups during the processing campaign justify long-term testing for most of the new applications. Fast degradation of diluted sugar-containing streams leaves no alternatives to testing of continuous process with fresh supply of feed material ("feed-and-bleed" operation mode). Batch experiments may be applicable only for analysis of permeate and possibly screening of various membranes. Properties of some membranes may vary during the service life due to cleaning, irreversible fouling, abrasion, compaction, etc. It is important to correctly estimate the mode of failure for various membranes and evaluate its potential effect on the production process. An example of potential operation problem revealed in the long-term studies is discussed below.

Two lab-scale elements of CeraMem membranes were installed in series. CeraMem membranes are a relatively new product characterized by very high membrane surface-to-volume ratio. Typically, the transmembrane pressure (TMP) is a good indication of membrane fouling. Figure 5 shows a standard fouling curve for any system with constant permeate flow. After the TMP was correlated with the feed pressure (Figure 6) it was discovered that the TMP increase was caused by plugging of feed channels between two modules in series with fibrous material. Using two and more modules in series in the design of industrial installations is the common practice. The channel plugging problem could not have been discovered in either batch or short-term continuous studies. This example illustrates the necessity to observe and closely monitor the pilot plant operation in order to reveal and eliminate potential repercussions.

CONCLUSIONS

1. Possible applications for membrane technology in beet and cane sugar industry have been reviewed. The sugar industry appears to be a large potential market for membrane technology.
2. Review of data obtained by various researchers indicated that micro- and ultrafiltration membranes can provide high quality permeate and, therefore, improve sugar quality and downstream processing parameters. It is emphasized that no significant purity rise should be expected across either micro- or ultrafiltration range membranes.
3. Use of membrane processes prior to chromatographic separation appears to be an ideal combination for both beet and cane juice purification. No purity rise, only suspended solids removal is required as a result of membrane filtration.
4. The necessity of serious long-term testing is emphasized for projects involving serious capital investment.
5. Involvement of an end user at the early stage of process development is critical for success of the project. Integration of membranes into existing plants will require the cooperative effort of membrane manufacturers, OEM's and end users.

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Table 1. Conditional classification of membrane processes.

Membrane process	Approximate Molecular Weight	Range of particle or molecule sizes	Example
Microfiltration (MF)	500,000-2,000,000	1-5 micron	Suspended and colloidal particles
"Loose" Ultrafiltration	300,000-500,000	0.05-1 micron	Smallest bacteria and viruses
Ultrafiltration (UF)	10,000-300,000	0.01-0.05 micron	Proteins
Nanofiltration (NF)	200-10,000	0.001-0.01 micron	Salts, sugars, ions, etc.
Reverse Osmosis (RO)	Less than 200	Less than 0.001 micron	

Table 2. Typical analysis of beet diffusion juice (Southern Idaho).

Component	Concentration		Molecular weight
	% on DS	% on non-sugars	
Sucrose	87.75	n/a	342
Invert sugars	1.03	8.59	180
Raffinose	0.42	3.5	595
Betaine	0.31	2.58	117
Citric acid	0.73	6.09	210
Malic acid	0.36	3.00	134
Lactic acid	0.12	1.00	91
Acetic acid	0.25	2.08	60
Oxalic acid	0.29	2.38	126
Other organic acids	0.20	1.67	--
Calcium, Magnesium	0.35	2.92	24-41
Sodium, Potassium	2.01	16.76	23-40
Proteins	**	--	15,000-100,000
Dextrans	0.3	2.50	50,000-2,000,000
Pectins	**	--	20,000-400,000
Glutamine*	0.7	5.84	146
Other amino acids*	0.7	5.84	100-300
Unaccounted non-sugars	1.26	10.50	--
Total non-sucrose	12.00	100.00	--
Total solids	100.00		--

* Concentration of glutamine and amino acids is calculated based on molasses content of about 9% on non-sugars.

** Information was not available.

*** Calcium and magnesium are calculated based on hardness level of 12 meq/100 DS.

Table 3. Juice purity increase as a function of non-sugar elimination.

Juice purity	88	
Non-sugars, % DS	12	
Non-Sugar Elimination, %	Permeate Purity, %	Purity Increase, Points
5	88.53	0.53
10	89.07	1.07
15	89.61	1.61
20	90.16	2.16
25	90.72	2.72
30	91.29	3.29
35	91.86	3.86
40	92.44	4.44
45	93.02	5.02

Table 4. Expected purity increase at 10% non-sugar removal.

Juice purity	82	83	84	85	86	87	88	89	90
Purity rise @ 10% NS elimination	1.50	1.44	1.37	1.29	1.22	1.15	1.07	0.99	0.91

Table 5. Experimental data on purity and color changes across the MF and UF membranes.

Feed Material	Membrane Pore Size	Change Across Membrane		Reference Number
		Purity increase, points	Color decrease, %	
Cane clarified juice	300 kD		10-15	(12)
	6-20 kD	0.3-1.3	30-40	(2)
	0.05 micron	1.5		(19)
Cane molasses	0.2 micron	0.3-0.8		(21)
Affination syrup	15 kD	1.2	40	(18)
	300 kD	0.6	21	
	0.1 micron	0.6	7	
Beet raw juice	15 kD	1.5		(5)
	15 kD	1.7		(4)
	20 kD	2.5-4.0		(2)
	0.2 micron	0.2-0.4	**40-50	(21)
Beet molasses	0.2 micron	0.3	40	(21)
Press water	0.2 micron	0		(21)

* kD=kiloDaltons, characterizes molecular weight cutoff (MWCO), e.g. 10 kD-10,000 MWCO

** Only for highly colored samples

Table 6. Pilot plant control strategy design.

Parameter to control or monitor in a pilot unit	Why this information is necessary or useful for a full-scale installation
Temperature	Affects viscosity and flux, may be critical for bacterial growth
Recirculation flow rate	Necessary for sizing recirculation pumps, valves, heat exchangers, pipes
Inlet and outlet pressures on the feed side	Provides good indication of channel plugging
Feed to concentrate (or permeate to concentrate) ratio	Stabilizes the volumetric concentration factor (or volume reduction factor)
Permeate and either feed or concentrate flow rates	Allows calculation of permeate flux as well as material balance.

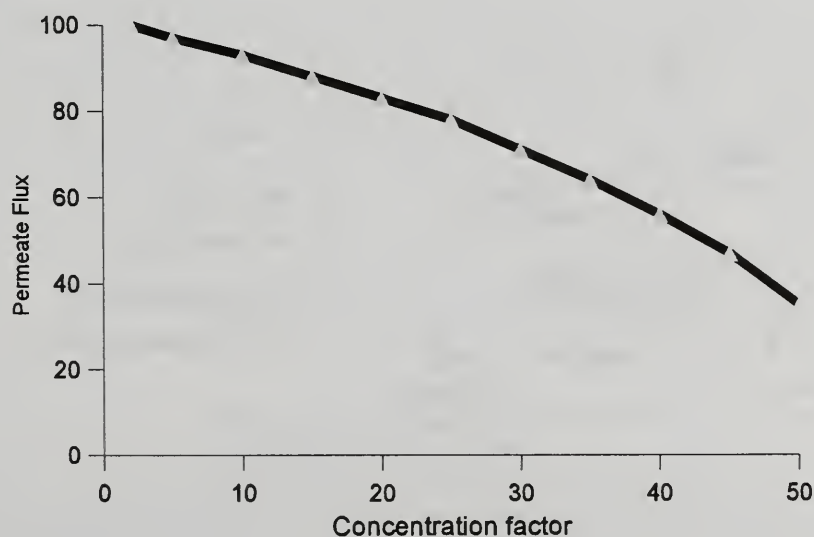


Figure 1. Influence of concentration factor (CF) on permeate flux (numbers are relative, 100% corresponds to flux at CF=1).

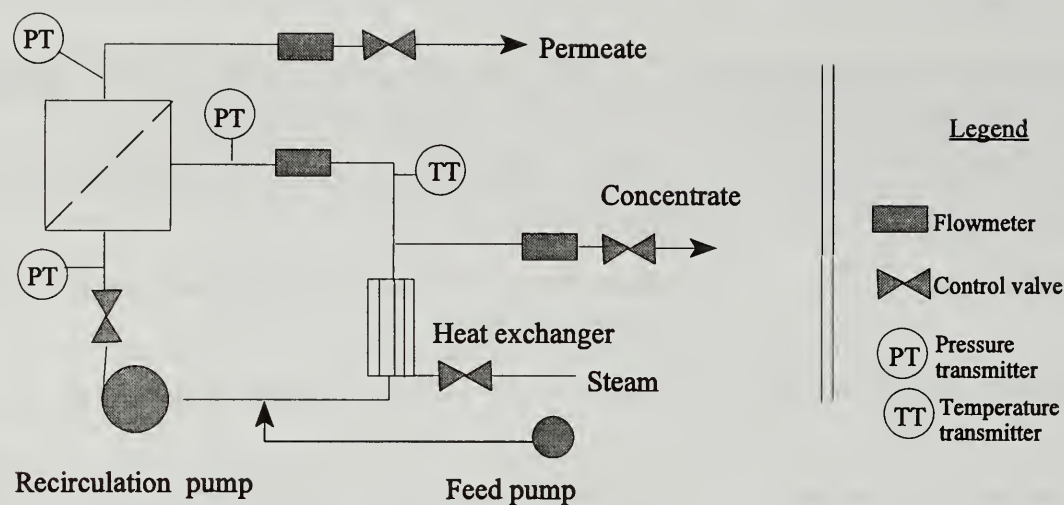


Figure 2. Membrane automation scheme maintaining constant permeate flow with permeate backpressure.

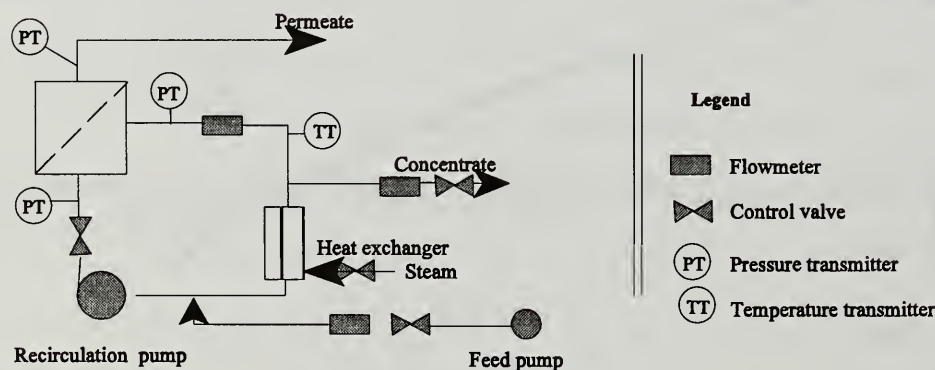


Figure 3. Membrane automation scheme with no backpressure on the permeate side.

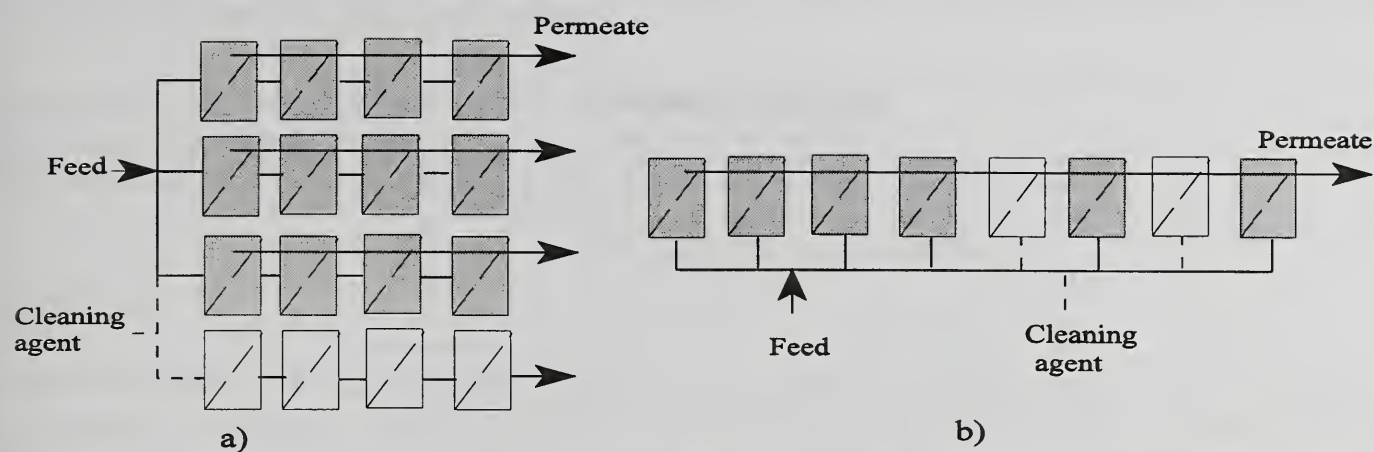


Figure 4. Sample layouts of industrial membrane system design.

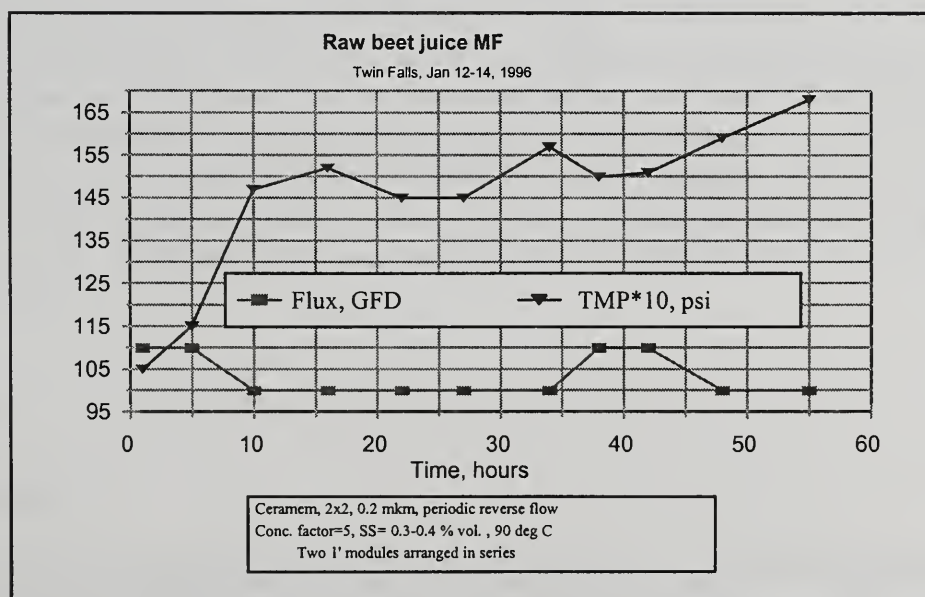


Figure 5. Microfiltration of raw beet juice.

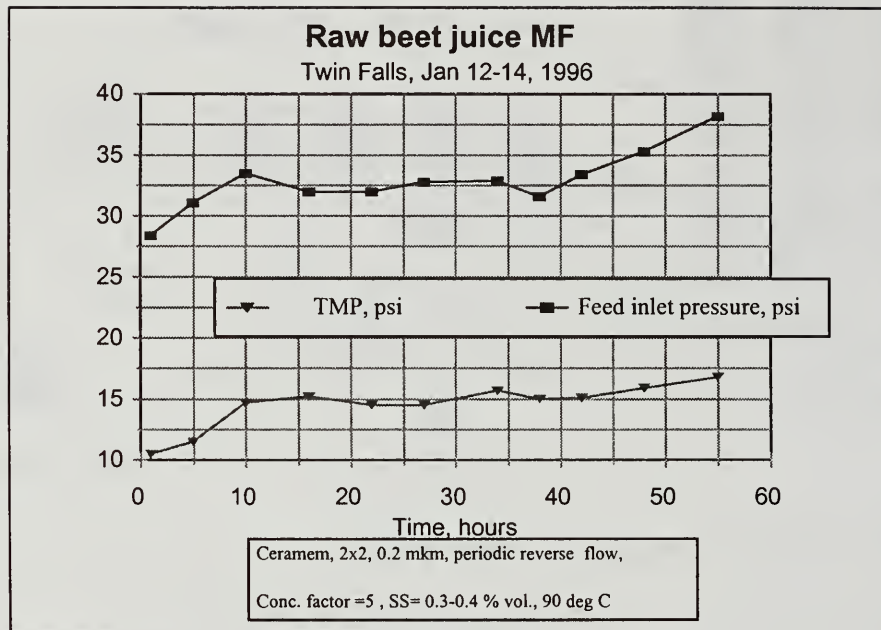


Figure 6. Feed pressure vs. transmembrane pressure (indicates that rise in TMP is caused by channel plugging).

NANOFILTRATION AS AN INDUSTRIAL ALTERNATIVE FOR RECYCLING USED BRINE FROM SUGAR DECOLORIZING RESIN

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SUMMARY

In the sugar industry, the development of ion exchange resin technology is restrained by the problem of regeneration waste disposal since the regeneration effluent contains a high concentration of sodium chloride and colored organics. In the present work, the recovery and recycling of waste brine previously used for decolorizing sugar liquor has been investigated using cost-effective nanofiltration process. The main objective of this project was to integrate an economically feasible nanofiltration system into the cane sugar refining process allowing brine recovery while reducing pollutant waste discharge.

INTRODUCTION

In the sugar industry, ion exchange resins are mainly used to remove colorants from cane sugar liquor. The regeneration of the resins is carried out by passing an alkaline brine solution through the resin to desorb colorants. The resulting resin regeneration waste characterized by high salinity and chemical oxygen demand (COD) is particularly polluting which increases disposal problems. In the present work, a nanofiltration process using cost-effective organic spiral-wound membranes was investigated for the treatment of resin regeneration effluent to recover spent brine for reuse while reducing the discharge of waste. The analytical characterization of the effluent allowed selection of the proper fraction of the effluent that could be efficiently treated by nanofiltration membranes. The performance of the nanofiltration membrane was experimentally evaluated on site in terms of productivity and quality of the permeate that would be recycled as brine, and effluent volume reduction in the retentate. A process combining nanofiltration and diafiltration was proposed to facilitate the final retentate treatment.

BACKGROUND

Nanofiltration Process

Nanofiltration is a relatively new pressure-driven membrane filtration process falling between reverse osmosis and ultrafiltration which has significantly widened the application of membranes in liquid-phase separations (1). Presently, most of commercially available nanofiltration membranes are negatively charged. While large inorganic and organic molecules with molecular weight greater than 300 Daltons are retained by nanofiltration membranes because of their size, ionic species are selectively retained according to their charge densities. This means that monovalent ions in salts, such as sodium chloride, tend to permeate nanofiltration membranes and

salts containing multivalent anions, such as sodium sulphate, are highly retained. Actually, nanofiltration mechanism is based on the concept of molecular exclusion as well as on the principle of electrostatic repulsion.

Previous Work on Sugar Decolorizing Resin Regeneration Waste Treatment

Ion exchange resin process is currently considered one of the most efficient methods for sugar liquor decolorization (2). High-molecular weight sugar liquor colorants such as melanins, melanoidins, products of alkaline degradation of sucrose, caramels and polyphenols are first adsorbed onto the resins, and finally desorbed from the exhausted resins using an alkaline 100 g/l sodium chloride solution at approximately pH 12. Effluents resulting from this regeneration contain mostly sodium chloride (up to 100 g/l) and important amounts of colored organic matter, and, therefore, constitute a major pollution source.

New environmental regulations having strong constraints have forced the sugar industry to develop economical innovative approaches to reduce the amount of waste solutions for disposal. In this context, different methods for spent brine waste reduction were investigated on a laboratory scale:

- a. Oxidations of color bodies to carbon dioxide by calcium hypochlorite, as well as chlorine gas were found to be inefficient and extremely expensive (3).
- b. Ozone was also used to oxidize colored compounds. However, incomplete oxidation and subsequent recombination of broken down organic matter into colorants prevented further study (4).
- c. While carrying out a first regeneration at low sodium chloride concentration and a second one at normal sodium chloride concentration, Bento differentiated two types of colorants according to their anionic affinities to the resins. Only the effluent resulting from the second regeneration could be precipitated with lime. After filtration, the solution containing mostly non-anionic colorants was satisfactorily reused to regenerate the loaded resins (4,5). Although a promising 60% reduction in effluent volume was reported, this process cannot be easily integrated into an ion exchange plant because of space requirements and need for expensive lime sludge equipment.
- d. The potential of crossflow filtration to treat regeneration effluent was investigated by several workers (6,7,8). According to Wilson and Percival, ultrafiltration induced a 45% reduction in organic matter in the portion recycled as regenerant, which represents 40% of the whole effluent (6). This low organics retention suggests that ultrafiltration membranes are not tight enough. Furthermore, the productivity aspect had not been approached by these authors.

Using tubular organic nanofiltration membranes, nanofiltration process was recognized as technically feasible on a pilot plant scale and demonstrated a 30% reduction in effluent volume and a 60% reduction in salt consumption at Hulett's Refineries (7,8,9).

MATERIALS AND METHODS

Pilot Plant Equipment

On-site pilot-plant tests were conducted using a 2.5-inch diameter by 40-inch long module on a pilot plant. The experimental set-up is shown in Figure 1. The retentate flow rate and pressure could be varied by manually operating valve V_1 that was downstream of the membrane. The permeate and retentate flows could be monitored by flow meters installed respectively in the permeate and retentate lines.

The regeneration effluent was provided by a sugar refinery. The effluent composition will be thoroughly studied later.

Experimental Method

Prior to nanofiltration, the effluent was filtered through a 10 and 5 μ cartridge safety filter to eliminate any potential suspended solids that could damage the membranes.

Spiral-wound organic membranes were selected since this configuration is largely applied to streams characterized by both low viscosities and suspended solids (10). Despite the alkalinity of this effluent, the spiral-wound type was selected as its operating costs are generally three times lower than those of the tubular configuration (9).

Operating nanofiltration conditions were set according to the manufacturers' recommendations. Applied pressure across the membranes was maintained between 15 and 20 bar. Temperature of feed solution was kept at 30°C. Because membranes have limited pH-resistance, the pH of the feed solutions was lowered from 13 to 11 by using hydrochloric acid.

As the regeneration effluent is not continuously discharged, it is more suitable to operate in a batch mode. The on-site pilot plant was first set in a total recycle mode which means that both permeate and retentate were returned to the feed tank, during 20 min. Batch concentrations were then performed by continuously extracting permeate and recycling retentate. Diafiltration was performed on the final retentate by continuously replacing extracted permeate with process water.

The volumetric concentration factor (VCF) was determined as follows:

$$VCF = \frac{V_f}{V_r}$$

where V_f represents the volume of feed (1), V_r the volume of retentate (1).

After completion of the experiment, membrane cleaning was carried out by first rinsing with water, then circulating a 0.02% alkaline solution for 60 min. When more than two days had elapsed between runs, membranes were stored in 0.1% solution of sodium bisulphite to inhibit microbiological activity.

Analytical Method

Composite permeate and retentate samples were collected during the operation of the nanofiltration system, at regular time intervals.

The optical density (OD) was chosen as a measure for the colorants concentration in the chemically complex industrial effluent. The similar evolution of OD and COD during a typical regeneration cycle shown in Figure 2 confirms that colored matters are the main polluting organic species. Organic pollution abatement was therefore characterized in terms of color removal. The optical density was measured at 420 nm using a Perkin-Elmer spectrophotometer.

Chlorides and COD analyses were performed by the Institut de Recherches de l'Industrie Sucrière (IRIS) according to their standard protocol.

The membrane performance was characterized by the capacity to recover sodium chloride (Rec_{NaCl}), and remove colorants (Rem_{Col}) in the composite permeate. Both terms derive from mass balance considerations:

$$Rec_{NaCl}(\%) = \frac{[NaCl]_p * V_p}{[NaCl]_f * V_f} * 100 = \frac{[NaCl]_p}{[NaCl]_f} * \left(1 - \frac{1}{VCF}\right) * 100$$

$$Rem_{Col}(\%) = \left(1 - \frac{OD_p * V_p}{OD_f * V_f}\right) * 100 = \left(1 - \frac{OD_p}{OD_f} * \left(1 - \frac{1}{VCF}\right)\right) * 100$$

RESULTS AND DISCUSSION

Characterization of the Regeneration Effluent

The nature of the regeneration effluent was preliminarily investigated to select the fraction that was the most suitable for treatment by nanofiltration. Effluent samples were collected at 9 m³ intervals during a regeneration cycle. Figure 2 displays the evolution of the composition of the effluent that had passed through the resin vessels, in terms of sodium chloride concentration, COD and OD. It is seen that initially COD and OD increase much faster than sodium chloride concentration. Table 1 shows that Fraction 1 situated between 0.8 BV and 2.1 BV contains 93% of the total sodium chloride and 50% of the total COD present in the effluent (1 BV=28 m³ in

this study). In order to recover a salt permeate concentration high enough for recycle, nanofiltration work is focused on this effluent portion which represents 1.3 BV per regeneration.

As a result, a 600 l volume of composite effluent taken at 9 m³ intervals from 0.8 BV to 2.1 BV during the regeneration cycle, and, therefore representative of the average composition of the treated effluent, was processed for all runs.

Effect of Time on Flux Performance (Total Recycle Mode)

In a batch mode, the volumetric concentration factor (VCF) effect is tied to the time effect so that one can estimate which causes membrane fouling. Consequently, a total recycle mode experiment was first made to determine the typical effect of time on the permeate flux. The plot of permeate flux versus time is showed in Figure 3.

Regarding membrane selectivity, sodium chloride and colorant retention was respectively estimated at 3.7% and 96.4% at the beginning of the experiment, and at 4.1% and 97.1% after 270 min. Therefore, these consistent results are in agreement with the expected retention of the membrane used which retains large organic molecules and allows the passage of monovalent ions.

Flux Performances in Batch Concentration Runs

Nine batch concentration runs were conducted to evaluate the permeate flux according to the operating VCF. It should be emphasized that initial optical densities of feed vary from 4.7 to 29.3 (Table 2). The strong disparity in the industrial effluent composition is mainly caused by variations of effluent sampling operation. In addition, influent sugar color, service run length, and regenerant dosage may also induce fluctuations in industrial effluent composition. However, these on-site experiments enabled us to cover a wide colorants concentration range.

A plot of permeate flux versus time and VCF is represented in Figure 4. Despite the variations of the feed composition, the curves present overall a similar trend: permeate flux decreases as time and VCF increases, and therefore, the optical density of the retentate increases. Maximum effect on flux occurs by 3 hours, after which there is little change.

VCF 15 was achieved in the batch mode which means that the initial effluent volume can be reduced by 93%. From those graphs, it is reasonable to deduce that permeate flux is strongly affected by the concentration of colorants. The permeate flux was therefore plotted against the optical density of the retentate for the 9 nanofiltration runs (Figure 5). The flux decreased significantly as retentate colorant concentration increased.

Salt Recovery and Colorants Removal in the Permeate

Table 2 summarizes the calculated colorants removal and salt recovery in the permeate obtained for each batch experiment. Uniformly high colorants removal varying from 95.4% to 99.0% is achieved such that the optical

density of the composite permeate remains below 1.1, whereas 75.9% to 94.5% of the salt is recovered, depending upon the final VCF value. As expected, the salt recovery in the permeate increases with increasing VCF. Therefore, one needs to find a compromise between product rate which decreases as VCF increases, and, recovery. These results demonstrate that, in Run 26 for instance, the nanofiltration process allows the recovery from regeneration effluent waste, of 93% brine permeate at a 93 g/l sodium chloride concentration while eliminating 95% of the colorants in the permeate. At the same time, 50% of the pollutant species generated during regeneration are concentrated in 7% of the volume. This reduction in effluent volume would facilitate secondary treatment of the regeneration effluent.

Sodium chloride and sodium hydroxide were added to the permeate to restore the regenerant components to their original concentration and pH. It was verified on lab scale that the efficiency of brine permeate and fresh brine to regenerate loaded ion-exchange resins was similar.

Treatment of Final Retentate

The final retentate at VCF 15 collected from Run 26 was processed through the nanofiltration membrane in a diafiltration configuration. It was observed that the permeate flux remained constant as long as process water replaced extracted permeate since the optical density of retentate remained constant throughout the diafiltration. Once an amount of water equal to that of retentate had been introduced, the addition of water was stopped and the concentration of retentate was processed til VCF 2. This whole operation enabled to reduce the quantity of salt by 50% in the retentate as well as its volume by 50%. The subsequent biological degradation of the desalting retentate containing 50% of the COD should be easier in a waste treatment plant.

CONCLUSIONS AND PERSPECTIVES

The organic spiral-wound nanofiltration membranes tested in this study demonstrated satisfactory qualitative performances for the recovery of sodium chloride and the removal of organic matter from the regeneration waste, in the permeate. Nanofiltration process allowed to achieve respectively a 90% reduction in salt consumption and a 93% reduction in water consumption for brine fabrication while reducing the volume of waste discharged from sugar refineries.

Nanofiltration separation process proved to be economically efficient to retain raw sugar colorants including both low and high molecular weight molecules.

Decline in permeate flux rate due to membrane fouling was observed in both total recycle and batch concentration experiments. Membrane fouling increased for the first 3 hours and stabilized.

The final VCF was set at 15, generating a good compromise between permeate flux, salt recovery and effluent volume reduction. The treatment of retentate was completed by combining nanofiltration and diafiltration which induced both volume and salt concentration reduction.

The proposed system for effluent treatment makes the decolorization resin technology more economically attractive although the polluting charge remains constant since nanofiltration consists simply of separating brine from organic matter. In cane sugar refining, this process should open up new perspectives such as the combination of ultrafiltration and ion-exchange resin integrating brine recovery by nanofiltration on raw sugar remelt.

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Table 1. Composition and distribution of the COD and sodium chloride between the different fractions for a regeneration cycle including a 28 m³ volume of decolorization resin.

Fraction	Volume (BV)	COD			NaCl		
		Concentration (g O/l)	Weight (kg)	Contribution (%)	Concentration (g/l)	Weight (kg)	Contribution (%)
1	1.3	7.8	281	50	88.2	3175	93
2	1.0	9.8	265	47	5.6	151	5
3	1.6	0.4	18	3	1.8	81	2

Table 2. Colorants removal (Rem_{COL}) and sodium chloride recovery (Rec_{NaCl}) in the permeate during batch experiments.

Run (n°)	VCF	OD at 420 nm			Rem_{COL} (%)	[NaCl](g/l)			Rec_{NaCl} (5)
		feed	retentate	permeate		feed	retentate	permeate	
6	4.1	4.7	11.4	0.2	96.8	52.6	54.5	52.8	75.9
9	6.5	11.1	67.3	0.2	98.5	80.1	80.4	79.0	83.4
12	5.4	10.3	36.4	0.2	98.4	75.7	78.8	74.9	80.6
14	8.9	19.1	116.0	0.5	97.7	83.3	83.2	83.4	88.9
22	5.8	24.1	130.0	0.2	99.3	93.1	81.8	91.5	81.3
23	8.0	24.1	184.0	1.1	96.0	93.1	79.8	89.1	83.7
24	15.0	29.3	75.5	0.3	99.0	70.5	76.1	71.4	94.5
25	15.0	14.3	94.5	0.3	98.0	63.6	63.3	64.4	94.5
26	15.0	8.2	134.4	0.4	95.4	98.1	90.7	97.3	92.6

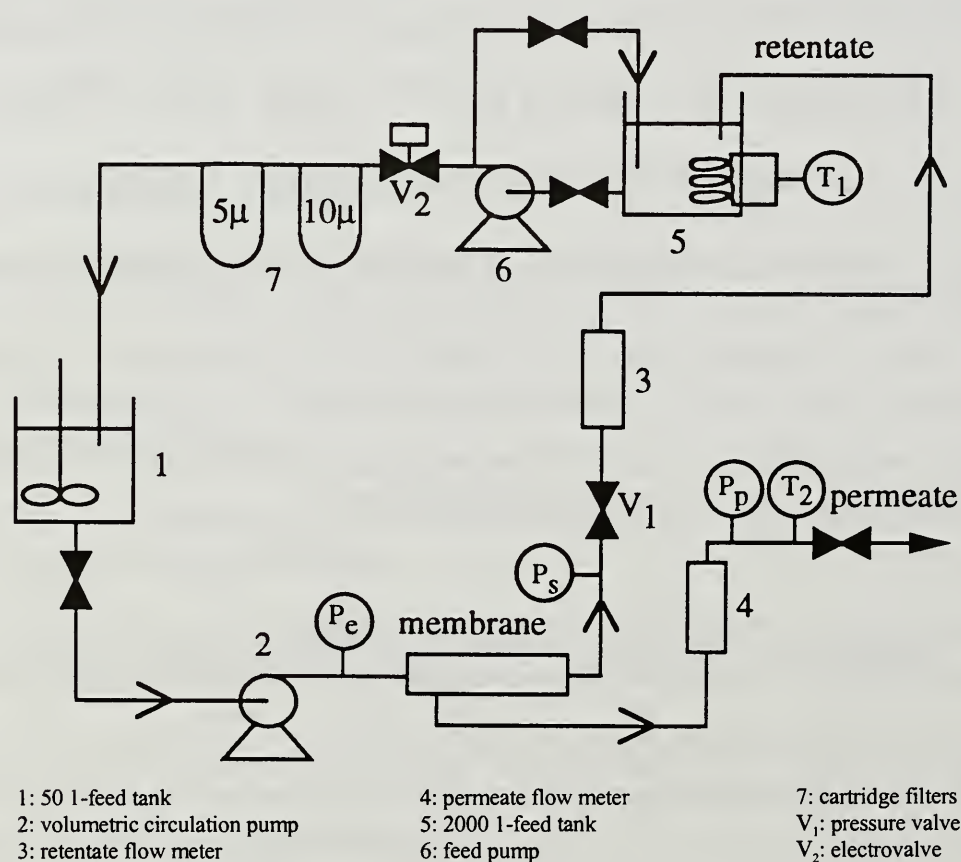


Figure 1. Schematic experimental apparatus for nanofiltration.

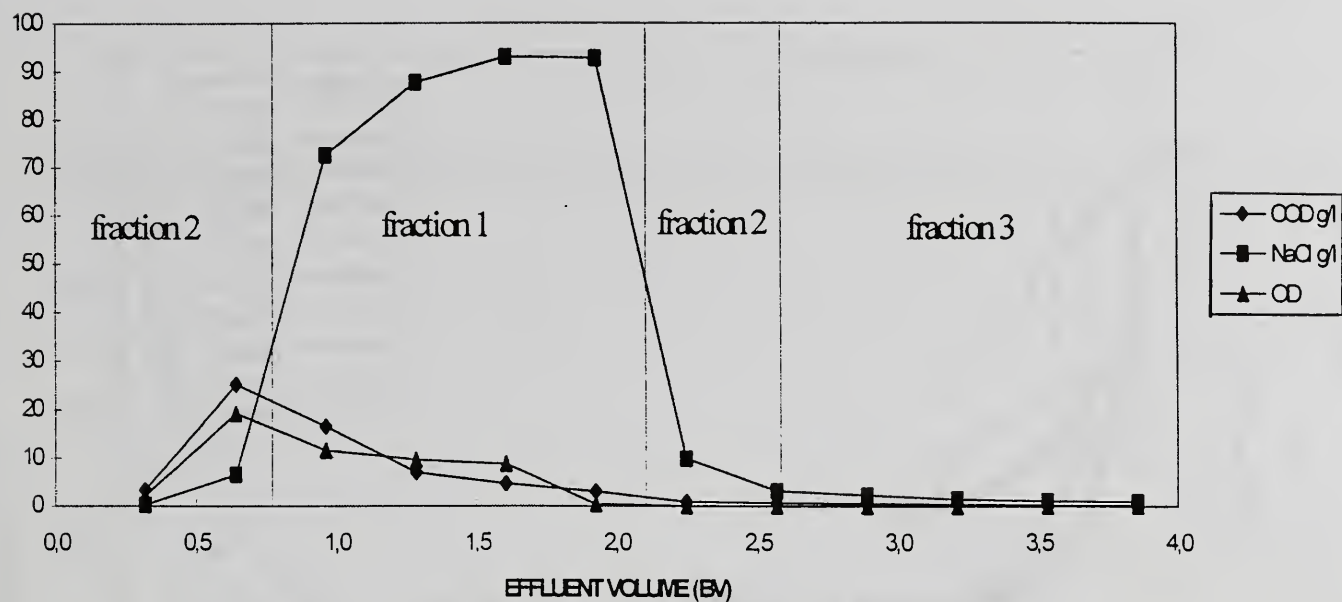


Figure 2. Regeneration profile as a function of effluent volume (BV) for sodium chloride concentration, chemical oxygen demand and optical density value.

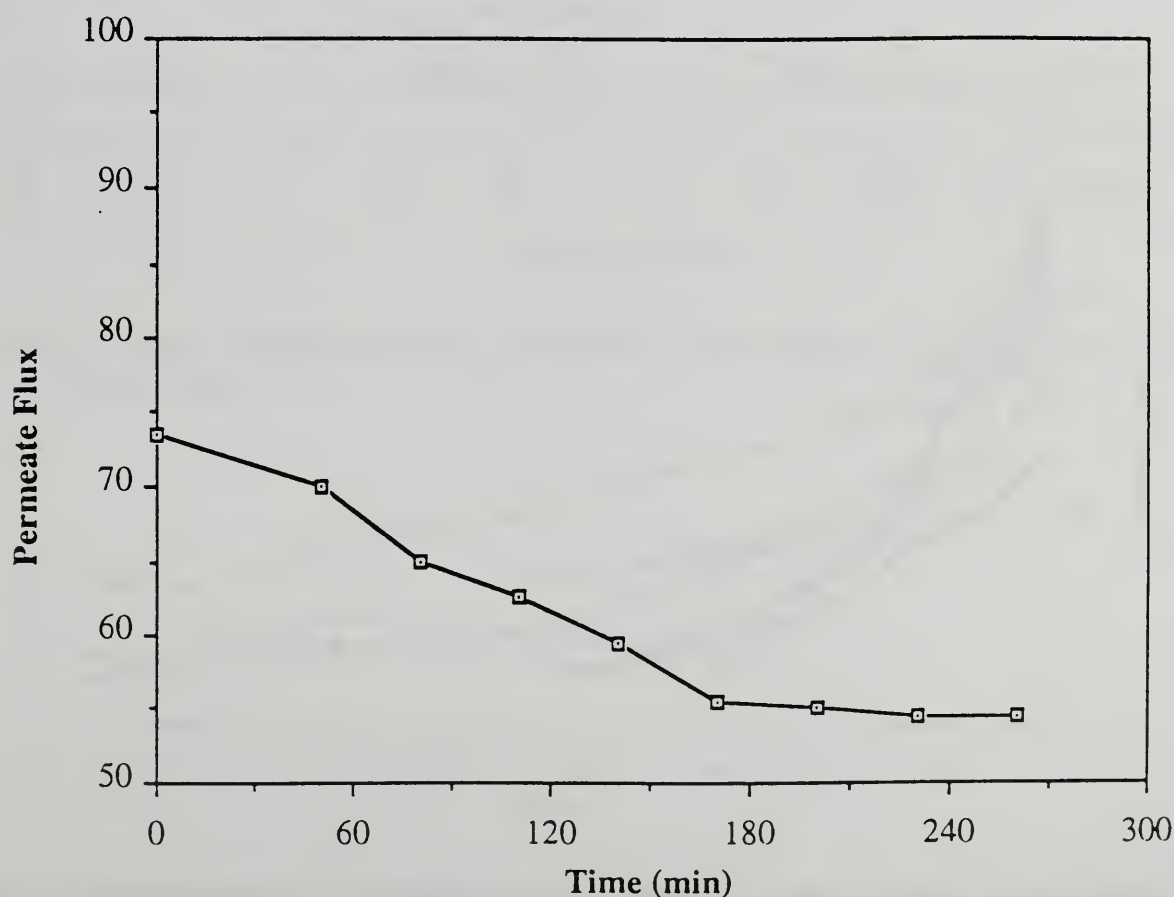


Figure 3. Variation of permeate flux as a function of time in total recycle mode. Operating conditions: OD of the feed=10.6, $T=30^{\circ}\text{C}$, $Q_c=600\text{ l/h}$ and $\text{TMP}=15\text{ bar}$.

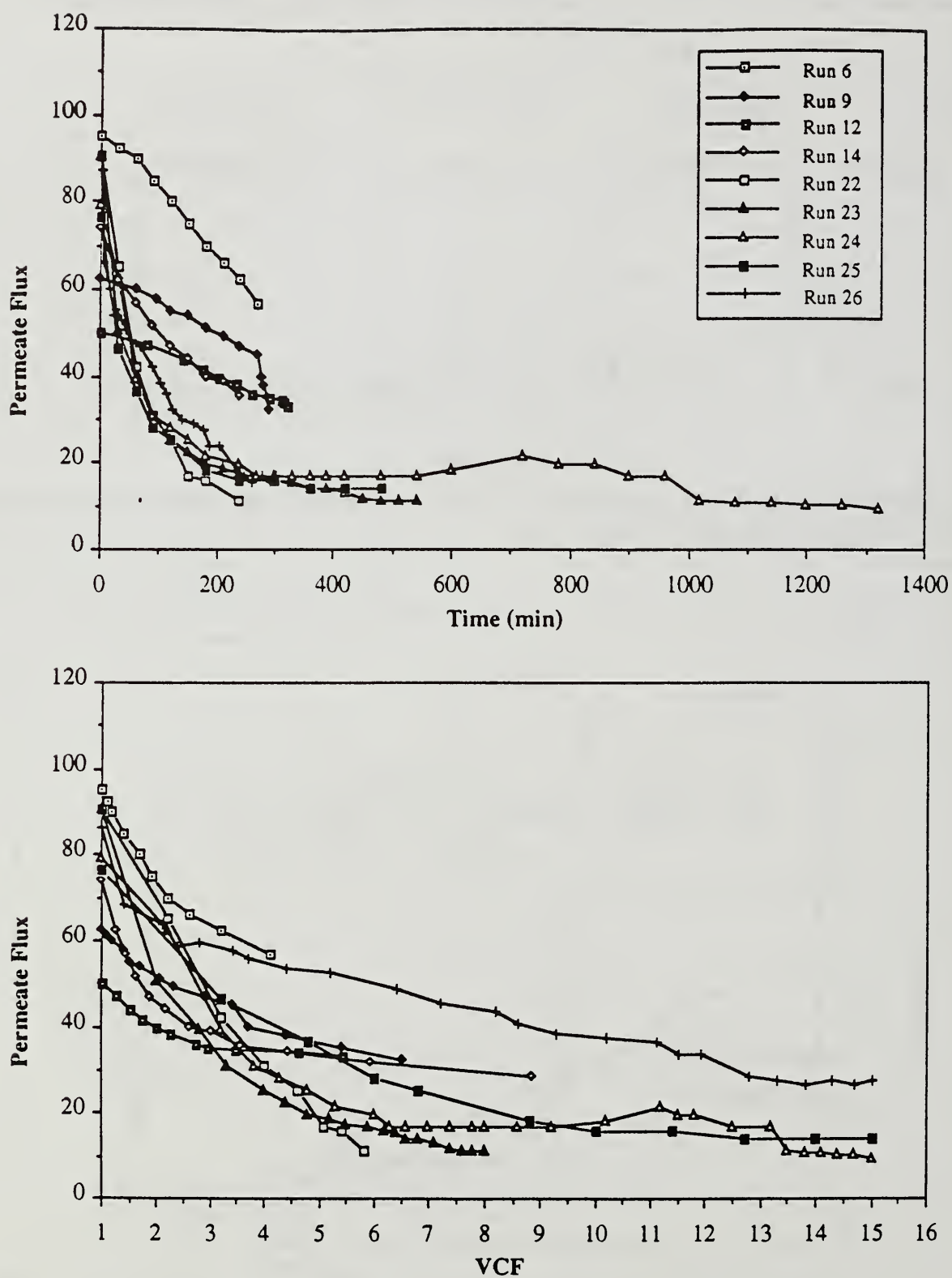


Figure 4. Variation of permeate flux as a function of time and volumetric concentration factor in a batch mode. Operating conditions: $T=30^{\circ}\text{C}$, $Q_c=600\text{ l/h}$ and $\text{TMP}=20\text{ bar}$.

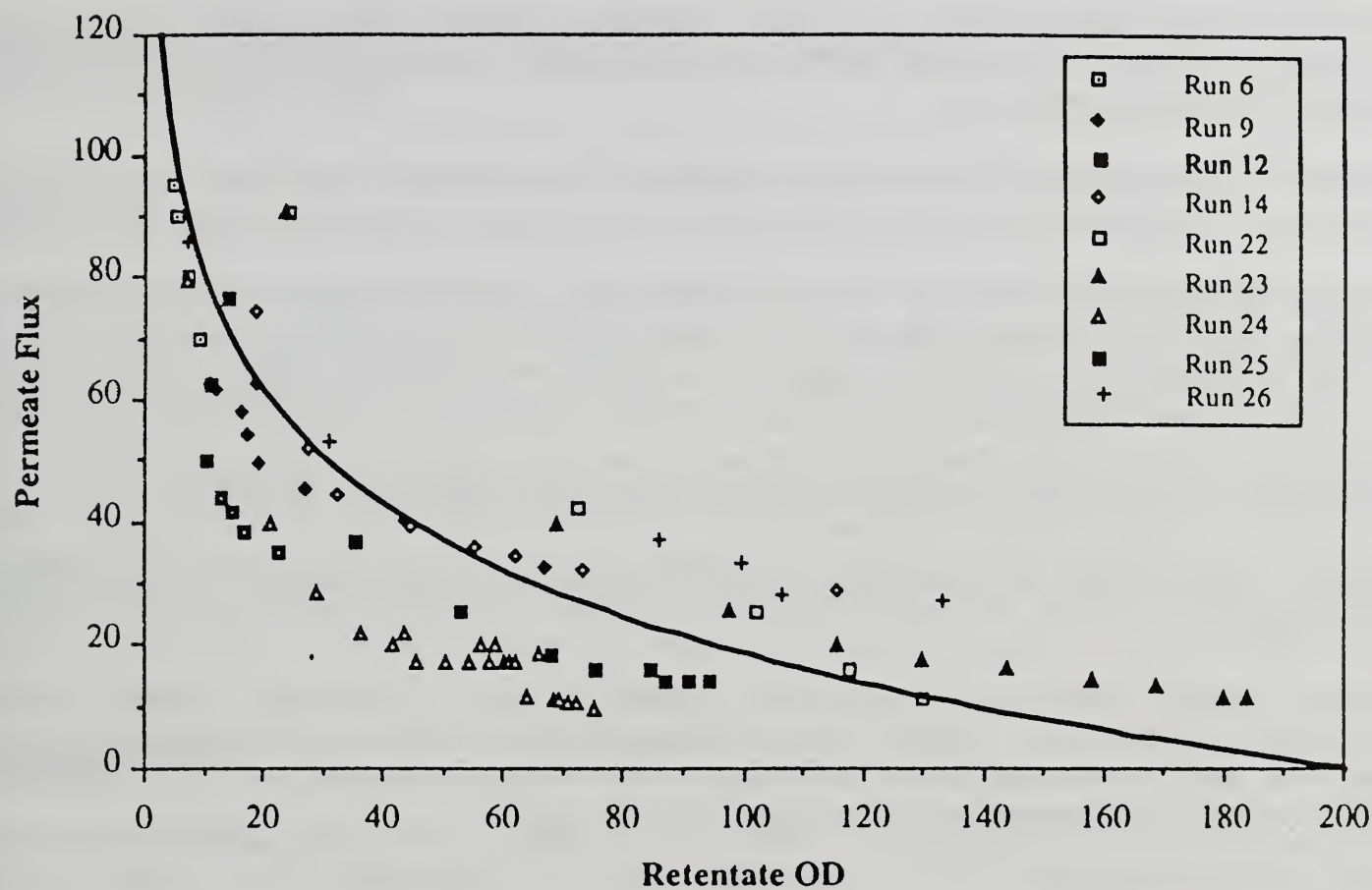


Figure 5. Permeate flux as a function of the optical density of the retentate. Operating conditions: $T=30^{\circ}\text{C}$, $Q_c=600\text{ l/h}$ and $P_{tm}=20\text{ bar}$.

DISCUSSION

Question: Can you tell us how salt recycling affects the resin?

Theoleyre: We have a similar efficiency of the resin. The system has been in use for six months now, and there is no effect on the color of the sugar and the work of the resin. Recycling salt does not decrease the decolorization efficiency of the resin.

Question: You mentioned the pH and temperature limitations of the membranes you are using. Are membranes available now, or do you see membranes coming, that will not have these limitations of temperature and pH?

Theoleyre: We are looking at that now. We already have that technology, and adaptation to new membranes will be very easy. We feel that in the very near future, pH resistant and temperature resistant membranes will be available. Pilot tests with that type of membranes are on the way. The question is what type of colorants are removed by the membranes and does the color build up. We are interested in the colorants that are removed by the membranes and interested in the analysis of those colorants. Even after six months we still see very good decolorization by the membrane. There is no decreased decolorization capacity of the resin.

Question: What is the upper salt concentration that produces too high an osmotic pressure? Or for an efficient reverse osmosis?

Theoleyre: Reverse osmosis is classically used to process sea water. In sea water treatment, the salt concentration is around 40 g/L. At 40 g/L of salt, the pressure for reverse osmosis membranes is about 70 bar, so that is our limit. It seems that the limit for reverse osmosis membranes is around 40-50 g/L, so that is not useful in our application because we want to go up to 100 g/L of salt.

SENSORY AND CHEMICAL PROPERTIES OF LIQUID SUGAR

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ABSTRACT

Liquid sugar may be manufactured from raw beet sugar without further crystallization. This makes the overall process of beet sugar manufacturing more efficient since the total loss during processing is reduced. The odor and flavor intensity of these liquid sugars are higher due to the lack of a purifying crystallization step. Efficient techniques to purify the solutions must be used. Different applications have different purity requirements.

In this investigation liquid sugar samples from Danisco Sugar AB's refinery in Arlöv, Sweden, were analyzed with GC-MS and GC-FID. These results were correlated with results from sensory analysis with multivariate techniques. The chemical-sensory correlation thus obtained may be used to reduce the off-odor and off-flavor problems occasionally developed during the liquid sugar manufacturing process. The aim of this investigation was to use this correlation to facilitate the removal of off-odor and off-flavor problems in the process.

INTRODUCTION

The use of liquid sugar instead of crystal sugar has proved successful in many industrial applications, as this product is easier to handle and to control the quality. Liquid sugar can be manufactured by dissolving white crystal sugar in water or by using process syrups from the refinery. In the latter process fewer purifying steps are needed. The use of this approach makes the overall process of sugar manufacturing more efficient since the total loss during processing is reduced. However, different food applications have different purity requirements and, since the odor and flavor intensity of the never crystallized sugar is higher, odors and flavors may be experienced in some applications. Most important, however, is to be able to control the levels of the odors and flavors.

There are many different causes for the development of off-odors and off-flavors in different production streams in a sugar refinery. The components found in beet sugar can be divided into three main groups: metabolites of the beet itself, compounds formed in the sugar beet as a consequence of microbial activity in the soil or compounds that are resorbed by the beet during growth, and finally, compounds formed during storage or in the sugar manufacturing process (1).

In earlier work, compounds giving rise to odors and flavors in beet sugar have been identified and quantified. However, the results from the GC analysis have seldom been correlated with the sensory analysis. Such a link between instrumental and sensory analysis is necessary to identify the chemical origin of odors or flavors in sugar and to evaluate the importance of various volatile compounds. In the present study volatile components in the liquid sugar were tentatively identified using head space GC-MS and quantified using GC-FID. A sensory panel was trained on the same samples, and a descriptive analysis of the odor and flavor was carried out. Finally,

the integrated peaks from the FID and the results from the sensory analysis were correlated with a Partial Least Squares analysis (PLS).

MATERIALS AND METHODS

Samples

Samples were kindly provided by Danisco Sugar AB from its refinery in Arlöv, Sweden. Samples with different odor and flavor profiles were needed. The sampling model described below allowed samples with different profiles to be produced.

The liquid sugar undergoes a series of purification steps on its way to the finished product. These steps include a mechanical press filter, a styrene adsorbent, a cation exchanger, and an anion exchanger. In our experiment, liquid sugar samples taken after the cation exchanger were further treated in an activated carbon column system according to figure 1. Samples were taken at points when a fixed amount of liquid sugar had passed the columns. Four different samples, A, B, C, and D, were taken. The sampling conditions are shown in Figure 1. Sample A was taken directly after an activated carbon column when 1.7 L sugar solution/g carbon had passed. Sample B was taken at the same time after a second column placed in series with the first one where the carbon had just been replaced with new carbon. Sample C was taken after the first column when 3.4 L solution/g carbon had passed, and finally, sample D was taken directly after the second column when 1.7 L solution/g carbon had passed. The sampling procedure was repeated about one month later and samples E, F, G, and H were taken, where E is equivalent to A in the sampling model, F to B, and so forth.

Analyses of °Brix, color, pH, ash content, and invert according to ICUMSA (International Commission for the Uniform Methods of Sugar Analysis) were carried out on site at the sugar refinery.

Instrumental analysis

In order to extract the volatile compounds from the gas phase above the liquid sugar, a dynamic head space method, modified from Hall, *et al.*, (2), was used prior to GC analysis. Every sample was analyzed three times. The GC detectors used were a Flame Ionization Detector (FID) and a mass spectrometer (MS). The response from the FID was used for integration of the peaks whereas the MS was used for identification of volatile compounds.

Sensory Analysis

The samples that were analyzed with GC-MS and GC-FID also underwent sensory evaluation. A panel of eight persons was used. The panel was trained on samples with odors and flavors that were typical of those found in the sugar solutions, together with the samples to be evaluated. Both sniffing and tasting were performed in order to allow evaluation of both odors and flavors. A list of suitable attributes was made and the evaluators reached consensus about the attributes and the intensity of the different odors and flavors. The intensities of the odors and

flavors of the samples were then evaluated on a scale from 0 to 100, where 0 corresponds to no odor/flavor and 100 to a very strong odor/flavor.

Analysis for Correlation between Instrumental Analysis and Sensory Analysis

The areas of the integrated peaks from the GC-FID analysis were analyzed for correlation with the sensory data, thus comparing the sensory quantifications of each attribute to the gas chromatographic data. For some gas chromatographic peaks the repeatability was not satisfactory, and thus these peaks could not be used in further analysis. Furthermore, the difference between replicates of the same sample was, for some attributes, too great compared to the difference between samples, and these attributes had to be ruled out before multivariate analysis.

A partial least squares analysis (PLS) was carried out on a dataset containing the remaining gas chromatographic and the sensory data. The sensory attributes 'total odor and flavor', 'dark syrup odor and flavor' (dark syrup is a commercially available product from Danisco Sugar AB, Sweden), 'chlorine odor and flavor', and 'rubber odor' were investigated as these were different between samples and exhibited good repeatability. The loading plots, where variables like the GC-peaks and sensory attributes are distributed in the x-y plane, were studied and the peaks that were situated closest to the sensory attribute in question were considered associated with that attribute.

RESULTS AND DISCUSSION

Sugar Analyses According to ICUMSA

The results from the sugar analyses according to ICUMSA are shown in Table 1. Brix, ash content, and color values were within the normal range. The invert was slightly elevated for the samples E to H, and the samples E, G, and H had pHs out of the normal range.

Sensory Analysis

The samples were analyzed with respect to 12 attributes, chosen and agreed upon by the sensory panel during introductory sessions. For some attributes there was no or almost no significant difference between the samples, and these attributes were therefore discarded before the analysis for correlation.

Analysis for Correlation between Instrumental Analysis and Sensory Analysis

The compounds associated with the different odors and flavors in the samples, according to the PLS analysis, are shown in tables 2-5. There was good concordance between the sensory evaluation of the odor attributes and the evaluation of the corresponding flavor attributes. This was true for all evaluated attributes and therefore odor and flavor attributes were evaluated together.

Not all of the compounds found to be associated with the sensory properties are considered to be odorous themselves, but most of them are. Dimethyl disulfide is reported to have an intensely onion-like, very diffusive odor. 2,6-Dimethyl pyrazine has a sweet, fried odor, resembling that of fried potatoes (3). 3-Ethyl-2,5-dimethyl pyrazine and 2-ethyl-5-methyl pyrazine have a roasted, and nutty odor (4). 6-Methyl-5-heptene-2-one has an oily-green, pungent, herbaceous, grassy, and diffusive odor (3). 2-Heptanone has a characteristic banana, slightly spicy, odor (5).

It is important to bear in mind that even though an individual compound has a totally different odor according to the literature, where the compound may have been isolated or dissolved in another matrix, the combination of odors in this particular matrix may give rise to the odors detected by the sensory analysis. Furthermore, most odorous compounds have different odors at different concentrations.

Multivariate analysis for correlation between GC-analysis and sensory analysis has one great advantage over GC-olfactory analysis, also known as GC-sniff, namely that the whole odor and flavor profile in the matrix is analyzed and compared to GC data. In GC-sniff the odors of the separated compounds are studied individually. The true aroma of a product is, however, related to the complex interaction of all volatile compounds within the matrix, not to the odor of individual chemicals. Furthermore sensory analysis is usually the method used for control of the quality with respect to odors and flavors.

CONCLUSIONS

Sensory analysis revealed differences with respect to the total odor and flavor, the dark syrup odor and flavor, the chlorine odor and flavor, and the rubber odor.

Partial Least Squares, PLS, analysis showed that GC peaks corresponding to volatile compounds may be associated with sensory attributes. This may serve as a link between the sensory analysis, the most used tool for quality evaluation of odors and flavors, and instrumental methods to characterize volatile compounds. The approach of correlating sensory analysis to instrumental techniques may be applicable for many other techniques.

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Table 1. Sugar analyses according to ICUMSA.

	Brix	Color	pH	Ash content	Invert
Sample A	62.8	9	5.8	0.004	0.533
Sample B	62.8	7	7.5	0.009	0.592
Sample C	63.0	8	5.5	0.003	0.680
Sample D	62.2	12	8.0	0.004	0.663
Sample E	64.0	27	4.1	0.001	3.152
Sample F	62.9	32	7.1	0.004	3.149
Sample G	63.9	18	4.2	0.001	3.841
Sample H	63.7	19	4.0	0.002	4.337

Table 2. Compounds associated with total odor and flavor.

Dimethyl disulfide
2-heptanone
Dimethyl pyrazine
2,6-dimethyl-4-heptanol
4-methoxy phenol
2,2-dimethyl decane
Saturated hydrocarbon
Ethyl-dimethyl pyrazine

Table 3. Compounds associated with dark syrup odor and flavor.

2,5-dimethyl furan
Dimethyl disulfide
2-methyl-3-heptanone
Dimethyl pyrazine
4-methoxy phenol
6-methyl-5-heptene-2-one
Saturated hydrocarbon
Ethyl-dimethyl pyrazine

Table 4. Compounds associated with chlorine odor and flavor.

Dimethyl pyrazine
2-ethyl-5-methyl pyrazine
2,2-dimethyl decane
Saturated hydrocarbon
Ethyl-dimethyl pyrazine

Table 5. Compounds associated with rubber odor.

Dimethyl disulfide
Dimethyl pyrazine
4-methoxy phenol
2,2-dimethyl decane
Saturated hydrocarbon
Ethyl-dimethyl pyrazine

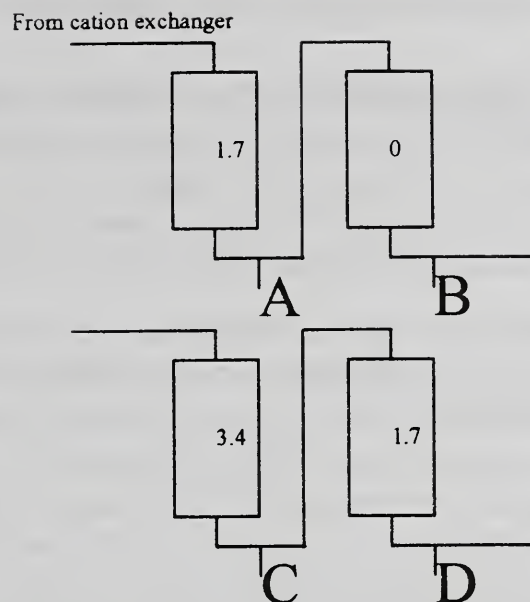


Figure 1. The sampling procedure. The boxes represent the activated carbon columns. Sample A was taken between the columns when 1.7 L of solution/g carbon had passed the first column and sample B after the second column, just after the carbon had been replaced with new carbon. Samples C and D were taken when another 1.7 L of sugar solution/g carbon had passed the respective columns.

DISCUSSION

Question: Are you now producing liquid invert syrup or liquid sugar syrups from white sugar? In Northern Germany, it has been traditional now for 30 years to use liquid sugar made from raw sugar. One big difference, I believe, between you in Sweden and people in Northern Germany, is that the Germans do not use SO_2 in juice purification, and maybe some of the off-flavors are coming from SO_2 usage.

Pihlsgård: Our liquid sugar syrups are mainly produced from raw beet sugar. It is possible that the use of SO_2 may increase off-flavors.

Question: We have also worked extensively on odors in beet sugar, and that type of work is not easy. When people are being asked to smell or taste a 60 to 70 Brix sugar solution, it is a tremendous challenge to the taster since sweetness tends to overwhelm everything else.

Did you not notice any of the volatile fatty acid odor complex? Also, it sounds as if you did not find any of the earthy odors often associated with sugarbeet.

Pihlsgård: In this study we did not identify any volatile fatty acids in our samples. However, some fatty acid like and earthy odors have been noticed in our liquid sugar samples.

Comment: This indicates that you were working with extremely pure sugar solutions, which makes the analytical and sensory work even more difficult.

BEET BREI ANALYSIS BY NEAR INFRARED SPECTROSCOPY

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ABSTRACT

This presentation examines the feasibility of near infrared (NIR) spectroscopic methods for quality analysis used for the beet payment to the grower. Another subject is examination for the feasibility of tare laboratory automation and chemical use elimination.

INTRODUCTION

The feasibility study for quality assessment by NIR Spectroscopy for beet payment was undertaken at Southern Minnesota Beet Sugar Cooperative tare laboratory. The study was done using a FOSS NIRSystems 6500 spectrophotometer. The data were based on reflectance spectroscopy of the beet brei from the tare laboratory.

METHODS

Conventional tare laboratory analysis was performed on the beet brei using a Rudolph Auto Pol II for % pol, flame photometer for Na⁺ and K⁺ and fluorescence spectroscopy for % α -amino nitrogen. An aliquot of the filtrate from the conventional analysis was used for % sucrose, raffinose, glucose, fructose and betaine by HPLC. The beet brei was prepared from whole, tared and washed beets. The production line has the capability of processing two samples per minute. Using the (NIR) cuvette or sample cup presentation, as several researchers (1-3) reported, the analysis time was prohibitive, one minute (4) per sample. The scanning time was reduced by changing the number of scans per sample from 32 to 16. This did not decrease the sample cycle time enough to make the method viable for tare laboratory operation. The largest time constraint was preparation of the sample in the one-quarter cup sample cell.

A stainless steel plate with a quartz window (2 1/4 inches by 2 1/4 inches) and sample compartment cover was fabricated to allow brei to be placed on the quartz window and scanned "on end" (5) from below. The brei was blended with a mechanical blender, and 25-30 grams of brei was placed on the quartz window. Scans per sample was set at 32. After the spectrum was obtained, the quartz window was wiped off. The sample rate was increased to one and one-half samples per minute, coming closer to the necessary speed requirements of two samples per minute.

Near infrared reflectance spectra were collected using FOSS NIRSystems NSAS Software and chemometric calibration equations were obtained using FOSS NIRSystems ISI Software. ISI Software gave better spectra management for identifying residual and spectral outliers in the development of the statistical model. Edye and Clarke (5) reported the same use.

NIR ANALYSIS OF BREI

Beets were taken from storage piles during the slice period and samples were processed through the tare laboratory to emulate the tare laboratory operation during harvest. Tests were run in November, December, January and February to obtain a wide variation of beet samples. The samples were very much like what we find in early campaign with a very narrow range of % pol. Our range of % pol was 14.80-20.40, with 95% of the samples being in the 15.50-17.50 % pol range; the calibration equations were not as robust as desired.

The cuvette or sample cell system gave an MPLS calibration for polarimetric sucrose with a correlation coefficient (R) of 0.970 and a standard error of prediction (SEP) of 0.343 %w/w. The cuvette or sample cell system gave an NIR predicted pol average of 17.282 and tare lab pol average of 17.252 on the same set of samples. The on-end sample system gave MPLS calibration for polarimeter sucrose with R of 0.981 and an SEP of 0.340%w/w. The NIR predicted average % pol was 16.842, and the tare lab average % pol was 16.997 using the on-end system. The slope of the calibration curve for pol using the sample transport system was 0.875. The results are presented in Table 1. The on-end system gave MPLS calibration for HPLC sucrose with R of 0.998 and SEP of 0.312%w/w. The NIR predicted average sucrose was 16.489 and the HPLC average was 16.590 for the on-end sample system for the same set of samples. The slope of the calibration curve using the on-end system was 1.362 for pol and 1.104 for HPLC sucrose. The results are presented in Table 2. The determination of the standard error of the conventional tare lab analysis was 0.123%w/w, and the standard error for HPLC was 0.164%w/w. A second derivative math treatment with a gap of six nm along with a small amount of smoothing was applied to all spectra used for calibration.

OBSERVATIONS

In this study, the NIR predicted HPLC sucrose has a better correlation coefficient and standard error of prediction than the NIR predicted polarimetric sucrose. The HPLC equation may have another advantage over pol during years when the beets are frost damaged during harvest and have to heal before being harvested. The sucrose value should be a better indication of recoverable sugar.

CONCLUSIONS

NIR spectroscopy does hold promise for use in the tare laboratory for beet payment. Sample cycle time is still an issue to be resolved. Instrument configuration may be able to be adapted for on-line use in the tare laboratory. Future work will be done to get a more robust calibration curve with samples of lower and higher sucrose content. Work will be continued to look at predicting α -amino nitrogen, betaine, fiber content, and other non-sugars. The object of the research is to develop an automated NIR quality assessment system for tare laboratory use. The system would need to be as good as the present system and be able to process two samples per minute. The system can be automated for labor savings. Reduction or elimination of consumable chemicals is a plus for cost savings along with environmental impact.

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Table 1. Calibration statistics for methods of determining pol % sucrose.

Method	R	SEP	Slope	NIR Predicted Average	Lab Average
Sample Transport	0.970	0.343	0.875	17.282	17.252
On-End	0.981	0.340	1.362	16.842	16.997

Table 2. Calibration statistics for the on-end system.

NIR Predicted	R	SEP	Slope	NIR Predicted Average	Lab Average
Pol % Sucrose	0.981	0.340	1.362	16.842	16.997
HPLC % Sucrose	0.998	0.312	1.104	16.489	16.590

DISCUSSION

Question: Can you give reasons for the better correlations you obtained with the instrument on the end compared to the core sample transport system? You certainly got better standard errors of prediction and the overall correlation coefficients were so much better.

Kingstrom: We feel that it is possibly due to the packing of the sample cell and the wider scanning area, the smaller sample area and more compressed sample.

Question: Can you comment on the sample thru-put you have achieved with this system compared to the previous system?

Kingstrom: We feel we could only process one sample per minute with the sample cell. With our modified sample presentation, we could process one and one-half samples per minute. However, this is still not within our time frame of two samples per minute.

Question: I have a question about the color of your beet brei sample. One of your slides showed very nice white beet brei color, but usually it develops a dark color very rapidly, which may have an influence on the results.

Kingstrom: That was simulated beet brei. I should have commented on that. It is not normally that white. This was only for taking a picture.

Question: Have you noticed any influence of the color on the calibration? And if so, how do you tackle the problem?

S.P.R.I.

Kingstrom: I have not noticed any effect of the color on the calibration. We used wavelengths 1100-1125 for developing the equations. We are not comfortable with picking out wavelengths.

Question: You aim to use NIR for payments. Then, in my opinion, you will have to determine the standard error of prediction. What would be an acceptable level?

Kingstrom: We are going to work on that and look more closely at the calibration equations, and look at specific wavelengths more closely. We used a lot of the software defaults.

Question: But what should be the acceptable level for payment? Should the standard error of prediction be 0.1 or 0.2? It is around 0.3 now.

Kingstrom: Possibly closer to 0.2.

Comment: That should be achievable. Four or five years ago, we were able to make calibration curves, and the standard error of prediction at the time was 0.1 to 0.5.

Kingstrom: We feel that we can get better calibration equations and correlation, and we are also looking at the sample process time.

ANALYSIS OF MOLASSES CHROMATOGRAPHIC SEPARATION STREAMS BY NIR

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ABSTRACT

The chromatographic separation processes used in the beet sugar industry for the separation of sucrose from molasses have advanced significantly during the last 15 years. Developments in chromatographic separation have seen the movement from batch separations to continuous separations based on both simulated moving bed systems and sequential systems. Systems in use today separate two or three components. Instrumentation used on chromatographic separators typically consists of conductivity detectors and on-line refractometers. This paper describes the use of on-line NIR for the determination of sucrose, RDS, betaine, and solution absorbance. It shows the work completed on the determination of calibrations for these substances, and provides examples on the use of data available from an on-line NIR spectrophotometer.

INTRODUCTION

The conventional process for the extraction of sugar from sugarbeets is not capable of extracting all of the sugar; an economically substantial portion of the sugar ends up in the residual molasses syrup. In the past 15 years, chromatographic separation processes for recovery of sucrose have been developed and commercialized. The process American Crystal Sugar (ACSC) uses for the recovery of sucrose from molasses is known as "continuous simulated moving bed chromatography".

Physically, the system consists of eight connected cells having feed, water feed, and product valves on each cell. During a complete cycle of the system, molasses is added to one cell for 8 to 12 minutes while water is added to another cell. Simultaneously, sucrose and the salt-rich fraction are removed from a third and a fourth cell.

During operation, a separation profile is established. That profile is moved through the system using an internal recycle flow of about 500 gpm. As this profile passes the appropriate valve, either extract or the salt fraction is removed from the system. Due to the movement of the profile through the system, it is necessary to switch the position of feed and water additions as well as the positions of product and salt extraction. Those step changes occur approximately every 10 minutes, with all steps in the process of the same length.

On-line process measurements made at ACSC include conductivity and RDS. The solids measurement is made with an on-line refractometer installed on the transfer line leading from Cell 4 to 5. Figure 1 shows a plot of the conductivity and RDS. In addition to the on-line measurements, the laboratory measures sugar content, solids, conductivity, and color. The laboratory measurements are used to measure system performance. The combination of data from on-line instrumentation and laboratory analysis is used for setting process variables.

Process parameters that can be changed include feed rate, ratio of salts to extract, water to feed volume ratio, step time, and internal recycle rate of the system. These operational parameters are changed based on the shapes of the circulation brix and the conductivity curves of the system. Information on sucrose concentration and the concentration of other components was not available in real time when the system was originally installed.

Laboratory measurements of refractometric dry substance (RDS), sucrose by polarimetry, and color by spectrophotometry result in time delays of hours, whereas the time between sample collection and report of analysis for sucrose and betaine is in the order of days. When it comes to making adjustments in tuning parameters, the time from implementation of a change to observing a change in system performance may be 80 minutes or more; therefore, analytical results for sugar by polarimetry and solids by refractometry with time delays measured in hours are acceptable. Typical time delays of a day or more needed for off-site analysis relegate that information to the historical category, and probably are not useful for control purposes.

As has been previously reported, NIR can be used for the simultaneous measurement of sucrose, betaine, and RDS of the separation profile in the chromatographic separator (1). That work was completed in order to provide on-line information for adjustment of operating parameters of the chromatographic separation. Additional work has been completed on the measurement of specific analytes in the feed and product streams. Specifically, sucrose, purity, betaine, RDS, and color have been measured in the sucrose-rich product or extract stream. Feed molasses has also been analyzed for sucrose, RDS, and betaine.

MATERIALS AND METHODS

An NIRSystems (Foss, Silver Spring, MD) Model SY-4500-P scanning NIR spectrophotometer equipped with a transmission pair probe was used for process measurements in the Molasses Desugarization Plant at East Grand Forks, Minnesota. The system was equipped with a flow-through cell fabricated from commercially available valve components. Spacing between the probes is approximately 0.8 mm. Other probe transmission gaps have been used successfully in this application. The gap between the transmission pair is easily changed; probe spaces of 0.75, 2.5, and 4.5 mm have been used successfully. All NIR measurements were made at process temperatures of 80°C.

Flow to the cell is continuous, with sample scans completed and the spectral data averaged across the time interval needed to obtain the NIR scans. Spectra are averaged and recorded with data transfer to the Rosemount system each minute. During a 1-minute sampling sequence, 25-28 spectra are recorded and averaged. Total spectral sampling occupies about 45 seconds of each minute. Changing instrument control parameters can readily vary the number of spectra recorded during the minute. The analytical value generated using NIR will reflect the average process stream composition during the time period over which the spectra are collected. Calibration samples are collected during the time that the instrument is collecting spectra, thereby assuring that the analytical sample correctly reflects the NIR sample.

Measurements on all samples use wavelength scans from 1100-2500 nm. Depending on the process stream analyzed and the transmission pair spacing, a reduced set of wavelengths may be used for calibration development with 32 scans made on each sample. For calibration, data from the instrument was logged on

a laptop computer. Reference values were obtained using refractometry, polarimetry, spectrometric solution absorbency at 420 nm, HPLC, and ion chromatography. NSAS software from NIRSystems was used for data reduction using algorithms for partial least squares and multiple linear regression. On-line measurements are made using the NIR with the data sent to a Rosemount process control computer for trending. Since the Rosemount system allows storage of only the past 24 hours of previous data, archived data is stored on the NIR-Rosemount interface computer and imported into a spreadsheet for processing.

RESULTS

The original purpose of the work was to optimize sucrose recovery by providing information for adjusting process variables. It has been demonstrated that NIR instrumentation can be used to provide real time data on the separation profile in an SMB chromatographic separator. During a complete cycle of the chromatographic separator, concentrations of the analytes of interest vary in concentration as shown in Table 1.

Calibration for absorbance at 420 nm was also determined, but the precision of the measurement was not acceptable for process control or of analytical use due to the large range of values found in the separator profile: 3,000 to 175,000 ICUMSA units. A calibration for raffinose has been completed for the separation profile but has not been completed in the extract phase due to low raffinose concentrations in the extract and the low precision of the laboratory method at concentrations of less than 0.1%.

Calibrations for pol and purity were developed for the extract phase only. Pol values are known to be negative on some samples taken from the separator profile indicating optical activity due to components other than sucrose. Composite samples run throughout the year have shown that the bias between purity by pol and sucrose by IC is about 1.2% in the extract phase.

Figure 2 shows a calibration developed for the determination of purity and betaine. Figure 3 shows a calibration for RDS and 420 nm absorbance. The 420 nm calibration shows good agreement of the NIR and lab values. This is due to the narrower range of RDS and absorbance observed in the extract phase.

Previous work (1) was reported on the repeatability of the NIR as a measure of analytical precision. Those results are shown in Table 2. Measurement of the repeatability on extract completed for this work (Table 3) also indicates the excellent precision of the NIR method.

Use of pol and apparent purity as calibrations offers the advantages of ease and speed of implementation, plus the high precision of the analytical method. A calibration for sucrose and purity based on IC has been developed but was not used due to problems of the NIR verifying the IC values of the sampled stream. Often, the most challenging hurdle in the development of calibrations is related to obtaining reliable laboratory values in a timely manner, an observation that has been noted in the literature by others (3). In the case of pol and purity by pol, the values are readily available on site, and validation samples can be run on site using polarimeter and refractometer. Calibrations using pol and purity by pol work for extract, but they do not work with molasses and raffinate due to the higher levels of interfering compounds. IC or HPLC

sucrose-based calibrations must be used for these process streams. Calibrations for components in raffinate and molasses are being developed.

The physical design of the ACSC chromatographic separator includes header lines for the collection of extract and raffinate from the train. The source of extract in the header changes every 10 minutes as a new cell starts discharging extract into the header. The raffinate collection header operates similarly. The composition of extract produced by each cell in an operating train can be determined by analyzing the extract flowing out of the extract collection line. Figure 4 shows the on-line NIR analysis of extract purity on a sample drawn from the extract header. This analysis shows extract produced through three cycles of the system. There are differences between the purity of the extract produced from each cell, which may be related to the condition of the fluid distribution system and resin packing in the cell. The on-line NIR provides a means of analyzing the extract output and determining the cause of the observed differences. The purity trace shows a double peak, which occurs on every eighth peak, indicating that the cause of the peak is related to a specific cell in the separator. The effect is either related to the cell or is an artifact of the flow of extract into the collection system.

With the addition of product stream for NIR analysis, new applications for the information were suggested. Details provided by NIR on the extract phase from a chromatographic column may be used for system diagnostics. The condition of a cell's distributors or the packing of its resin may affect purity of the extract produced from any of the eight cells in a train. A method used for the measurement of the performance of a cell is to sample the extract phase of a particular cell for pol, RDS, and color. The sampling requires obtaining 11 samples spanning the start to the completion of the extract phase for that particular cell. A plot of the laboratory data is shown in Figure 5. This plot can be used for process optimization as a means of changing process flow rates to obtain better purity at lower color. Using NIR, it is possible to survey all cells in the train to determine if there are opportunities for performance enhancement. Figure 6 shows a plot of NIR analysis, with spectra averaged over a 1-minute period and results reported each minute. Use of a faster scanning spectrophotometer for the comparison analysis or higher frequency of reporting of analytical values will improve the peak shape shown in the figure.

The SMB system does an excellent job of separating sucrose from other components when operating on "virgin" molasses. Due to capacity limitations on molasses and extract storage at the MDS facility, it is necessary to process molasses containing non-sugars from extract-rich standard liquor in the sugar end. This sets up a recycle loop of non-sugars back into the chromatographic separator. Betaine is one of the non-sugars that is known to build up in the separator-derived molasses. Examination of the separator profile shows that the betaine concentration is increased in the extract phase due to the partial co-elution of the betaine with the sucrose in the extract fraction. Recycle of extract derived molasses back to the SMB separator will result in an increase in the concentration of betaine in the extract. Betaine is reported to have a negative impact on sucrose crystallization from low raw syrup (8).

The NIR can provide on-line information on the build-up of betaine in the extract. During the spring of 1997, the MDS plant was sending extract to the factory for processing and also processing factory molasses. Betaine concentration in the extract was determined with the NIR spectrophotometer. The NIR data were used to decide whether to process additional molasses from the East Grand Forks plant or to use virgin molasses from two other factories. Figure 7 shows the variation in betaine concentrations as the source of

feed was changed from virgin molasses to recycled molasses. In this case the absolute value of the analytical information was not of critical importance. The emphasis is on the change in the betaine concentration as the factory molasses was recycled back to the separator plant.

SUMMARY

At ACSC we have used NIR spectroscopy for the analysis of molasses chromatographic separation feed and product streams. This technique has the capability of doing multicomponent analysis and can be used for the determination of non-component specific analytical values such as purity and 420 nm absorbance in the extract phase. The technique may also have value for performing diagnostic analysis. The ability to determine the on-line purity of the extract phase can be used for diagnostic purposes. The purity profile of extract from a specific cell can be used as an indicator of separator cell performance. Other performance characteristics may be apparent from the on-line data. The on-line NIR analytical data is more informative than the traditional conductivity and RDS measurements

As has been shown in this work, on-line NIR analysis can be used to show the concentration profile of the specific components and provide the operator with information that can be used for process optimization. The analytical information provided by NIR has precision comparable to or better than the laboratory analyses, includes more components than available from the traditional lab, and provides the analytical values in real time. The NIR analytical data coupled with flow meters could be used to determine material balances around the separator.

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Table 1. Concentration ranges of components found in the SMB chromatographic separator.

Component	Feed	Extract Phase	Separator Profile
RDS	58 - 64	25 - 40	0 - 40
Sucrose	32 - 42 g/100 g	20 - 35 g/100 g	0 - 35
Betaine	2 - 8%	8 - 15 mg/ml	0 - 20
Purity (pol)	55 - 65	80 - 95%	-5 - 95%
Absorbance NWS @ 420 nm	NA	0.4 - 1.5	0.1 - 3
Raffinose	0.5 - 3%	<0.1%	0 - 1%

Table 2. Estimate of analytical precision.

Analyte	Laboratory Stdev	NIR Stdev	NIR Range
Sucrose	0.3	0.2	0 - 46 g/100 ml
Betaine	0.1	0.3 - 0.4	6 - 22 mg/ml
Absorbance	0.05	0.7	0 - 6 ABS
rds or DS	0.1	0.2	2 - 52 g/100ml

Table 3. Repeatability data.

	Dissolved Solids g/100 ml	Sucrose g/100 ml	Betaine mg/ml
range	27.3 - 28.7	28.5 - 30.4	7.4 - 8.7
avg	28.2	29.3	8.0
std dev	0.3	0.5	0.3
n	96	96	96

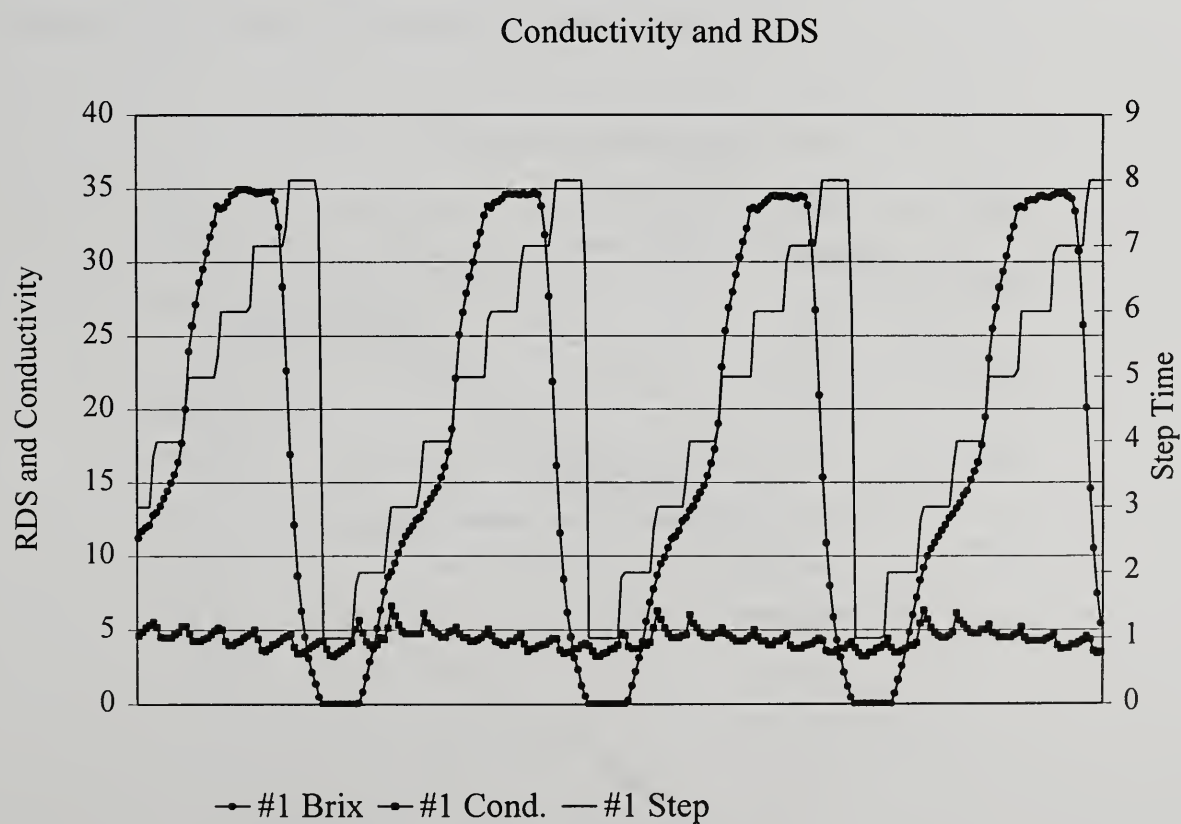


Figure 1. Recirculation RDS, conductivity and step time.

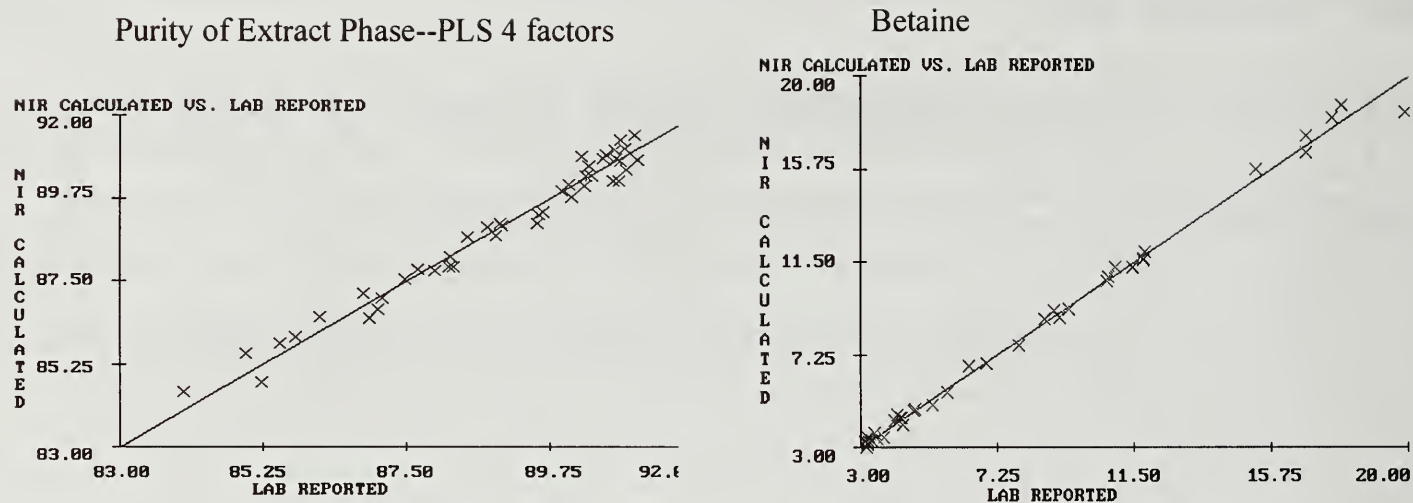


Figure 2. Purity and betaine calibrations.

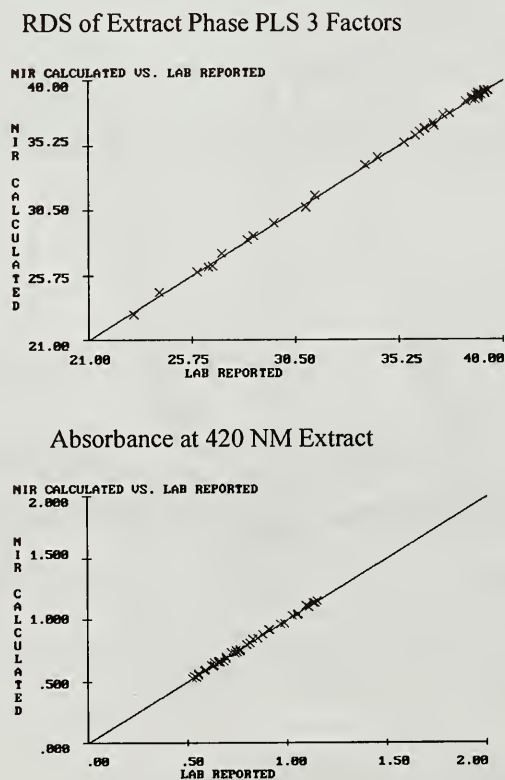


Figure 3. RDS and absorbance calibrations.

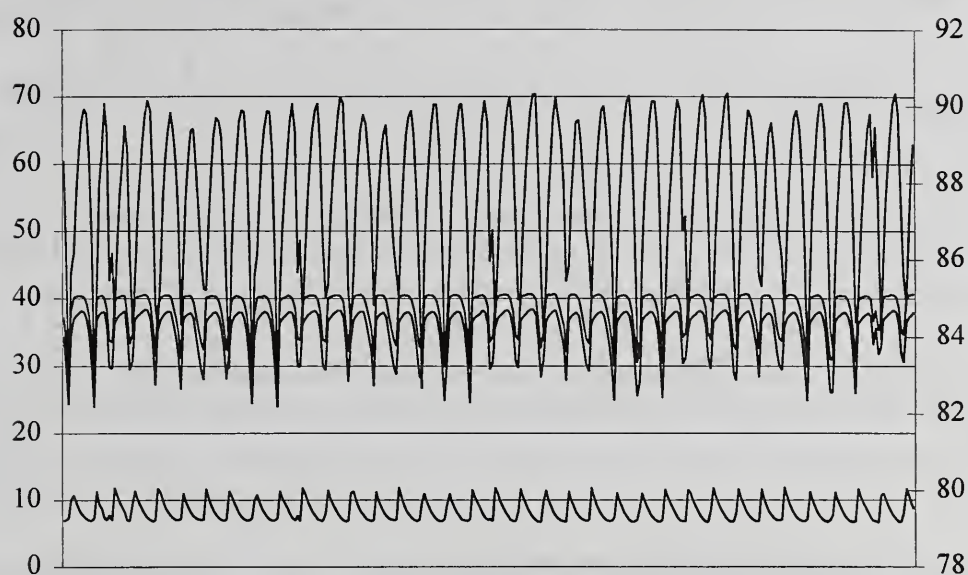


Figure 4. Extract purity, RDS, POL and 420 nm absorbance x 10.

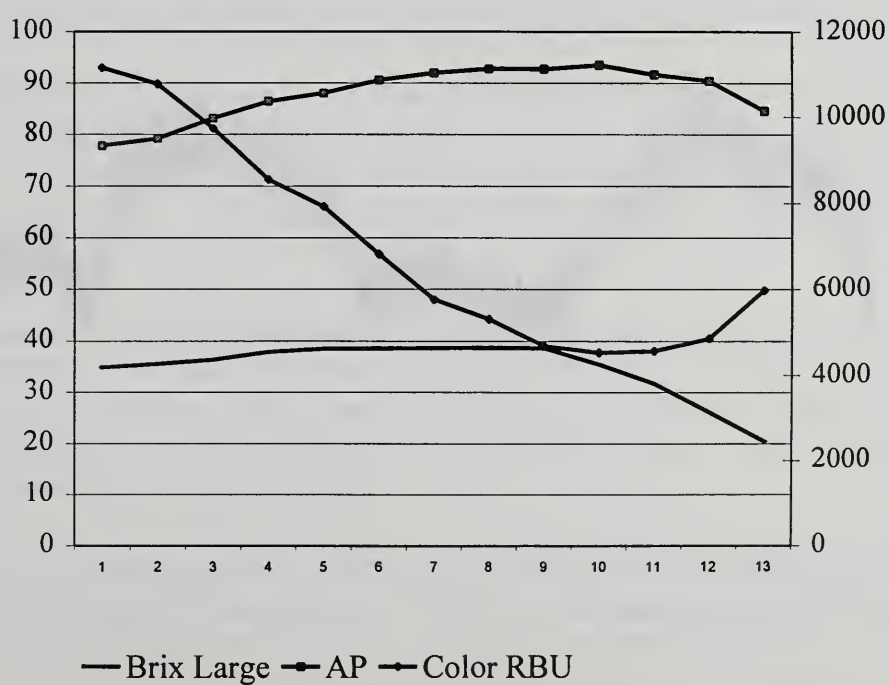


Figure 5. Laboratory values of "10 point pick."

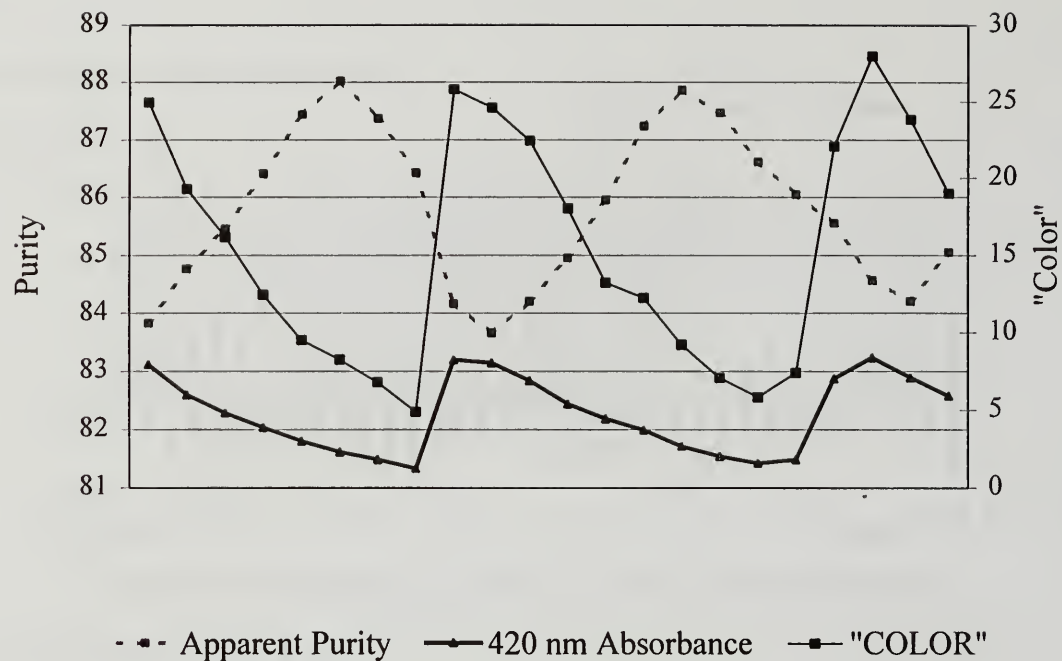


Figure 6. NIR analysis of extract phase 9/27/96.

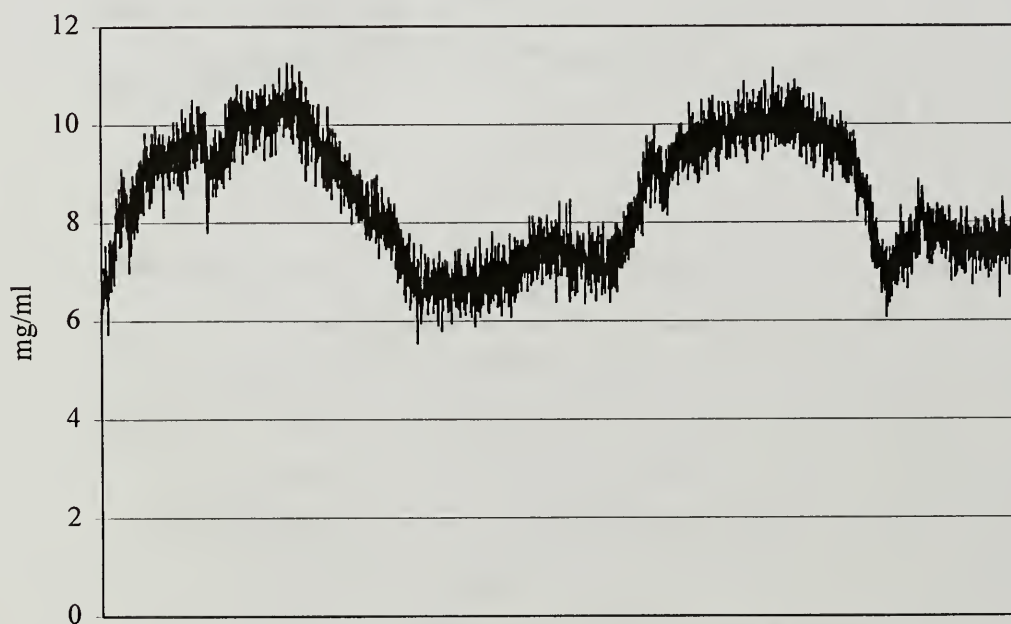


Figure 7. Betaine concentration, mg/ml in extract, 4/18/97-4/21/97.

DISCUSSION

Question: In your color analysis by NIR, what sort of error or sensitivity did you find? Are the color wavelengths near 420 nm or near 560 nm?

Wallevand: To answer the second question first, they are at 420, simply using the analysis of our spectrophotometer.

The calibrations for color are extremely sensitive. If you went outside the calibration set even by a small amount, the calibrations would tail off in one direction or the other. I do not have the specific numbers available, however. In other cases, we have seen extremely noisy looking spectra and values.

Extract (which is more homogeneous) seems to give more reproducible results in predicting color. Molasses and raffinate, being mixes, are difficult to fully characterize analytically. Molasses and raffinate vary so much in composition over the campaign and from factory to factory, characterizing them by color is meaningless to the NIR. NIR does not like ambiguity.

It may be that in streams whose “color” depends upon multiple chemical species whose contribution is constantly varying, the ability to predict quantitatively is confounded. The primary analytical method of measuring absorbance at 420 nm cannot specify the contribution of each colored component. We must keep in mind that when sugar chemists speak of sugar colors, they are not referring to the tight absorption bandwidths associated with a single inorganic species such as cobalt (II) chloride hexahydrate.

Question: What is the future of the traditional methods of analysis for the sugar industry?

Wallevand: One might think (incorrectly) that in an NIR environment, traditional analyses would go away. In terms of control of processes, we will be able to move beyond analysis and monitoring. It may be possible, particularly in the MDS plant, to actually operate the plant based on NIR. There are many industries that do this already — the petrochemical industry and the pharmaceutical industry are two examples.

As for the everyday analysis, NIR calibrations, as you know, are built upon extensive work using reference methods. Consequently, we will still need those particular methods to verify ongoing measurements with the NIR and update the calibrations. Right now at Crystal Sugar’s Research Center, we employ 2½ persons for 3 to 4 days each week to do QA and IC analyses for five factory labs. That work will not be diminished in the near term, but eventually it may change.

Question: First of all, I would like to compliment the authors on a very extensive piece of work in terms of the application of NIR and in showing how flexible the technique is and in terms of the very good calibrations.

Initially you showed the actual wavelength absorption for what you were looking at. I got the impression there was a very good peak around 2350 nm. I know with partial least squares, we can somewhat lose sight of what peaks we are looking at, what with it all being worked out for us in terms of factors and that sort of

thing. Have you been able to apply simple regression methods to see what peaks will pertain to those constituents you have looked at?

Wallevand: It seems that some of the betaine peaks were coming out in that area. Foss recommends cutting off somewhere around 2300 nm, maybe even sooner. We have looked very closely at the loadings and the weighting functions that are provided with the NSAS software. When there is a lot of noise in a region, I become skeptical about using those wavelengths. But I have gone somewhat beyond 2300. The instrument does go to 2500 nm. Because the spectra are very noisy in that area, we generally drop them off.

Question: Was your work standardized in terms of temperature, or is this not as critical? Lastly, from a quality control viewpoint, do you use your conventional system once in a while just to check on the NIR?

Wallevand: Initially, we planned to do more of this checking, but with all the other work we have to do, we have not been able to do as much as we would like. We are not running the MDS plant by NIR per se. A once a week calibration is indeed important for this kind of work. The quality of the predictions can deteriorate very quickly with NIR due to varying circumstances, such as varying quality of the beets and molasses.

Control of temperature is extremely important, although there are new methods, I understand, that can take into consideration temperature changes and adjust for them. The NIR is an excellent, albeit very expensive, thermometer. I have even done a little experiment in which I made a calibration based upon temperature changes within the cell and actually used the instrument as a thermometer.

Question: You use the NIR to monitor the plant. Do you have any problems utilizing the data obtained by NIR in real time to manage the plant?

Wallevand: The response time of an MDS plant is so slow that one may typically wait an entire day before adjustments to the system cause a perceptible change. Aside from mechanical problems that arise, the only variation in operation that should occur is the feed molasses. Timely adjustments made upon receipt of information from the NIR about the feed allay upsets in operation.

When molasses generated from the boiling of standard liquors containing extract is included as part of the feed molasses for MDS, a recycle loop of non-sugars to the chromatographic separator is set up. These recycled non-sugars are returned to the pan. The increasing contamination of the standard liquor by recycled non-sugars, particularly betaine, has a severely deleterious effect on sugar end operations. Especially when we are using this "house molasses", the operators know to switch from tanks of molasses having high betaine loading to other tanks that contain molasses from Crookston and Drayton (two of our factories that produce low calcium, non-extract derived molasses).

Comment: I would like to quickly comment. It is an excellent piece of work you have done. I think for SMB purposes, the relative position of the betaine is important rather than the absolute values.

Wallevand: Yes.

COOLING CRYSTALLIZATION APPLIED TO THE "EXTRACT" FRACTION OF A CHROMATOGRAPHIC SEPARATION PROCESS (SMB) OF MOLASSES

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ABSTRACT

Fractionation of sugar beet molasses by the Simulated Moving Bed (SMB) Ion Exclusion Process is in commercial use in many countries. The sugar-rich fraction (i.e. extract) obtained from this process is utilized in various ways during and after the regular beet campaign. Extract contains colorants which can complicate processes into which it is introduced. This can be handled by reducing the initial extract color using ion exchange resin, although this can create other problems. An alternative approach is a crystallization technique which enables the recovery of white sugar from highly-colored syrups. For the latter approach, we used cooling crystallization, as this has been successful with both highly-colored syrups and untreated raw juice. In our laboratory, a 3-stage cooling crystallization process was applied to extract. Commercial white sugar was obtained from the 1st crystallization step, and by recycling the crystals recovered from the 2nd and 3rd stages. Elevated levels of betaine and raffinose (both of which reduce sucrose solubility) enable the recovery of a molasses which was ~93% exhausted by the crystallization process. The crystallization data are presented, along with a working model describing the cooling crystallization process for extract in a sugar factory operating independently of the regular beet campaign.

INTRODUCTION

The chromatographic separation of molasses (sometimes referred to as "ion exclusion") has been used on an industrial scale for about 30 years (12). The efficiency of the process has been markedly increased by the recent introduction of the Simulated Moving Bed (SMB) technology (For reviews, see references 1, 2, 4, 5, 13, 14, 16). Currently, about 75% of sugarbeet molasses produced in the United States is processed via chromatographic separation using various systems built by different manufacturers (7).

The sugar-rich fraction (i.e., "extract") derived from ion exclusion typically has a purity of 86-92% and a color level 2-3 times that of thick juice produced by conventional beet processing. Extract can be processed in a variety of ways (6). For instance, during the campaign, it can be blended with thick juice and the resulting mixture boiled to recover sucrose. In contrast, during the intercampaign period, it can be directly processed to produce sugar without admixture of other syrups. The high color of extract necessitates modifications to sugar-end process cycles (e.g., raw sucrose and run-off recycling) to meet color standards in the final sugar product. Unfortunately, such modifications influence production costs which vary directly with extract color. This problem can be resolved by:

1. Decreasing extract color through the use of decolorizing resins;

2. Modifying crystallization processes to enable recovery of white sugar from highly-colored syrups.

The first approach, although technically feasible, is expensive; moreover, the waste streams produced during resin regeneration cycles make it environmentally unattractive.

An attractive solution for the second option is the use of cooling crystallization, which deviates from the traditional crystallization strategy based on water evaporation. Cooling crystallization for use in sugar processing was first explored by Mantovani, *et al.*, (9) and Vaccari, *et al.*, (19) who applied the technique to crystallization of sucrose from highly-colored thick juice. In their patent, Vaccari and Mantovani (17) used cooling crystallization to recover white sugar from standard liquor obtained without the usual recycling of raw sugars and highly-colored run-off syrups. The feasibility to recover saleable white sugar using 3 sequential thick juice crystallization steps was also demonstrated; reprocessing was required only for the raw sugar from the 4th crystallization step.

Cooling crystallization has the advantage of requiring less energy than conventional sugar recovery processes. It can produce commercial sugar with low ash and color using equipment that is less complicated than that used for boiling crystallization. Since it does not use decolorizing resins, no resin regeneration effluents are produced, making the process environmentally friendly. Cooling crystallization can be used for direct recovery of sugar from raw juice, thereby eliminating costly, conventional purification steps (20, 21, 22, 23, 24). This process was patented by Mantovani, *et al.*, (10), and has been applied on a small scale to cane sucrose crystallization (25). The economic and environmental benefits of cooling crystallization have spurred a cooperative effort by several members of the EC to further develop this technology (COPERNICUS Project; Ref. 3).

One drawback for implementation of cooling crystallization is that capital investments may be required for installation of equipment which may not be in place in existing sugar factories, although these expenses are outweighed by the benefits of the process. Moreover, given the variable nature of the starting syrups (esp. with respect to non-sugars and color loading), it is impossible to accurately predict the extent of process improvement afforded by cooling vs. conventional crystallization. Consider the following:

1. Cooling crystallization reduces the amount of mother liquor occluded within sucrose crystals. However, since this is influenced by parameters such as non-sugar composition, solution viscosity, crystallization kinetics, sucrose solubility, etc., the extent to which this will occur is difficult to predict.
2. During concentration and cooling crystallization, non-sugars (especially color precursors) can markedly affect juice colors. Moreover, certain types of colorants are preferentially occluded within sucrose crystals, regardless of the crystallization process used.
3. Sucrose cooling curves are dictated by crystallization kinetics which, in turn, are influenced by non-sugar composition.
4. The number of crystallization steps needed to produce molasses is determined by the total crystal yield. This is influenced by the non sugar composition and the solubility of sucrose in the particular system being studied.

Recently, we applied the cooling crystallization process to the recovery of sucrose from extract derived from the chromatographic separation of molasses. This was an interesting challenge, given the fact that extract has a non-sugar and colorant composition which is quite different in comparison with sugar feedstocks studied in the past. The results of this investigation are presented.

EXPERIMENTAL

Extract was obtained from the American Crystal Sugar Company, which has a molasses desugarization facility at its sugar factory in East Grand Forks, Minnesota (U.S.A.). This facility has a 525 tonnes/day molasses capacity and operates for 330 days/year. The desugarization process utilizes the SMB technology developed by the Amalgamated Sugar Company in Twin Falls, Idaho. The above extract typically has a Brix of ~68 and an apparent purity of 90-91. It is processed during the regular beet campaign and the intercampaign period as described above. Sucrose recovery from the extract is around 85-90%.

This investigation was carried out in two phases:

1. In Phase 1, a small amount of extract, the composition of which is shown in Table 1, was utilized to evaluate the characteristics of sugar which could be obtained from the solution by direct cooling crystallization.
2. Phase 2 entailed a complete series of cooling crystallization steps from extract to molasses. For this purpose, a second, larger quantity of extract was obtained from the above source. The composition of this extract (Table 1) indicates a higher purity than that used in Phase 1. Phase 2 extract also has more raffinose and higher (12%) colorant levels; it does, however, contain less betaine. These compositional variations may be due to differences in the starting molasses, and/or to a slight shifting of the separation band of the extract during the chromatographic separation process.

To carry out the crystallization trials, the system shown in Figure 1 was assembled. This consists of a 50 L-capacity thermostatically-controlled bath, a computer-controlled temperature programmer (E & F), cooling and heating elements (B & C), and a stirrer (D). Bath temperature is monitored via three probes (A).

Crystallization trials were set up by rotary evaporation of the feedstock to a pre-determined Brix level, followed by addition of seed crystals (avg. particle size: 10-20 μm). Crystals obtained by cooling crystallization were separated from the mother liquor using a laboratory basket centrifuge. Adhering mother liquor was removed from the crystals via affination with a pure sugar solution and washing with organic solvents.

The analyses of sugar crystals and mother liquors were performed using well-established, state-of-the-art methods: invert by the method of Berlin (15), betaine by HPLC (AMINEX HPX-87N), and raffinose using specific enzymes (Boheringer-Mannheim GmbH, Germany).

Morphological examination of sugar crystals was performed by viewing crystals in sucrose-saturated solutions using a stereoscopic microscope equipped with a camera (50-200x).

RESULTS AND DISCUSSION

Phase 1

In these trials, sucrose solubility data for extract were unavailable. However, given the high purity quotient (Table 1), it was assumed that the extract would exhibit characteristics similar to those of a pure sucrose solution. The solution was concentrated to a supersaturation of 1.1 at 80°C. After addition of seed, the temperature was gradually reduced to 30°C following a programmed, 5-step cooling profile (Figure 2). Since crystal growth kinetics for this system were unknown, cooling conditions utilized were based on prior experience with similar syrups, and were carefully implemented to avoid the formation of secondary nuclei. The use of milder cooling conditions during the initial and final crystallization steps was critical (11, 26), since the amount of sugar which can crystallize per unit time is less in these two stages. Indeed, at the outset, the available crystal surface is very low; by comparison, in the final stage (i.e., at low temperature), the kinetics of crystal growth are very low.

The product recovered by the above process is a commercial white sugar with the properties shown in Table 2. The sugar has a white brilliance that is normally observed with sugar recovered by cooling rather than by evaporation of water. The morphology of the sugar (Figure 3-A) is quite normal, even though elongation along the *b*-axis is less pronounced compared with beet sugar crystallized by conventional means. From extract with an initial Brix = 84.69, sugar recovery was excellent (59.35%). This exceptionally high yield can be attributed to the effects of non-sugars on sucrose solubility (see below).

Phase 2

Encouraged by the results from Phase 1, we next explored the possibility of using cooling crystallization to completely exhaust a larger quantity of extract following the scheme outlined in Figure 4. The cooling curves for the 2nd and 3rd crystallization steps paralleled the profile used in Phase 1 (Figure 2); however, the cooling times were increased 3- and 9-fold, respectively, to compensate (as is traditionally done in sugar factories) for the decrease in crystal growth kinetics due to elevated non-sugar levels.

The properties of the sugars obtained in Phase 2 are shown in Table 3. The characteristics of the white sugar are essentially identical to those of the white sugar from Phase 1 (Table 2); however, the crystal yield (64.97%) is higher compared with that from the previous extract trial. This is obviously a function of Brix value at the onset of crystallization. Interestingly enough, the properties of 2nd and 3rd product sugars are essentially identical; this is attributable to the prolonged cooling steps which improve the characteristics of the crystals. Clearly, however, the products obtained from the 2nd and 3rd crystallizations cannot be considered white sugars and require recrystallization via recycling.

From an inspection of Figures 3, it is clear that the morphology of the crystals undergoes a progressive modification in the 1st, 2nd and 3rd product sugars. This is due to the content and unique composition of the non-sugars present in the growth solutions which, besides inducing a progressive elongation along the *c*-axis, also cause a tapering of the left pole due to abnormal growth of the "*q*" faces and the consequent decrease in the "*p*" faces. The "twins" observed in the 2nd and 3rd crystallizations are Type 2 "twins", i.e., "twins" whose appearance is that of crystals grown in the presence of elevated levels of raffinose (18).

Figures 5-A and 5-B show "twins" in the initial and nearly final phases of development, respectively. Owing to the abnormal development of the "q" faces on the left poles, their shapes are quite unique.

The 1st and 2nd run-off liquors from the cooling crystallization exhibit only moderate increases in color and % invert; however, these substances are nearly doubled in the final molasses (Figures 6 & 7). The latter has a remarkably low sucrose purity (Table 4) due to the elevated levels of betaine and raffinose. Betaine and raffinose are known to decrease sucrose solubility (27, 8) and consequently help to raise the total crystallization yield to > 93.42%.

Using the results of our crystallization trials we developed a computer model for an integrated extract utilization process. The objective was the recycling of 2nd and 3rd product sugar to the starting extract to produce a "standard liquor" from which white sugar could be produced. Such recycling raises the purity and reduces the color of liquor sent to 1st crystallization, thereby improving the quality of the final white sugar product.

In this model, the process steps were optimized to compensate for the removal of sucrose by crystallization and for the resultant purity reductions in the run-off syrups. This was done to obtain the lowest possible molasses purity. In particular, the crystal/mother liquor ratio in the various phases was maintained at values which were compatible with centrifugation of the respective magmas. The calculations were refined until steady-state crystallization conditions were achieved. The mass balance data (Table 5 & Figure 8) show that, along with a high yield of crystal sugar, the quality of the latter falls well within the specifications for a commercial product, especially with regard to ash and color. From the data, we have a complete picture of the various parameters which should be followed to operate a hypothetical factory for processing extract. The model (Figure 9) provides for the continuous exhaustive crystallization of extract obtained from chromatographic separation of molasses, and includes parameters for the concentrations of the various juices, crystallization yields in the different steps and acceptable crystal/solution ratios.

The solution obtained by mixing extract with 2nd and 3rd product sugar is concentrated under vacuum in a continuous evaporator at 80°C to a pre-determined Brix value. After addition of seed, the liquor enters a small continuous evaporator in vacuo to complete the formation of crystals from seeds. Magma next enters a continuous vertical crystallizer. Commercial white sugar is recovered from the magma leaving the crystallizer by use of a continuous centrifuge. The resultant mother liquor is concentrated, then subjected to 2 additional sequential crystallizations as described above for the 1st product. In this way, 2nd- and 3rd-product sugar and molasses are obtained. The latter sugars are blended directly with diluted extract from the chromatographic separator, and the entire process repeated.

SUMMARY

The feasibility of recovering sucrose from extract obtained by molasses desugarization has been carefully examined. The data obtained from this investigation demonstrate that:

- Commercial white sugar can be recovered directly from extract by cooling crystallization.

- Crystals obtained from extract differ morphologically from those recovered by conventional beet processing; however, in terms of final product quality, this is not a problem.
- The yield of sucrose from cooling crystallization exceeds 93%.
- Molasses produced from extract by cooling crystallization has a very low purity.

Based on these results, a hypothetical model (Figure 9) of a factory for continuous, exhaustive crystallization of extract obtained from chromatographic separation of molasses has been developed.

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Table 1. Properties of extracts used in cooling crystallization trials.

Property	Phase 1 Extract	Phase 2 Extract
Brix	68.80	70.94
Polarization	62.05	65.08
Purity	90.19	91.74
pH	9.7	9.7
Invert %	0.11	0.04
Color I.U.	5530	6215
Raffinose %	0.35	0.45
Betaine %	2.45	1.50

Table 2. Characteristics of sugar obtained from the extract used in Phase 1.

Polarization	99.90
Color I.U.	27.27
Ash %	0.0057
Invert %	0.01
Crystal yield %	59.35

Table 3. Characteristics of the sugars obtained following the scheme depicted in Figure 4.

	White sugar	2 nd Product	3 rd Product
Polarization	99.90	99.00	99.00
Color I.U.	38.49	115	120
Ash %	0.007	0.015	0.0018
Invert %	0.010	0.011	0.012
Crystal yield %	64.97	62.66	49.45

Table 4. Molasses composition.

Brix	84.75
Polarization	35.86
Purity	42.10
Color I.U.	85400
Invert %	0.55
Raffinose %	3.76
Betaine %	12.55

Table 5. Mass balance.

	MASS	S	N	W	xxls	Brix magma	Purity solution	Crystal yield	xxls/ mother sol.
Standard (*)	100	70.97	4.87	24.16			93.58		
1st Crist.	30.09	23.84	4.87	10.37	47.13	87.97	83.04	66.41	1.21
2nd Crist.	17.38	8.95	4.87	3.56	14.89	88.97	64.76	62.46	0.86
3rd Crist.	9.92	3.54	4.87	1.51	5.41	90.15	42.10	60.45	0.55

(*) extract + 2nd and 3rd product

S = sucrose

N = non-sugar

W = water

xxls = crystallized sugar

xxls/mother solution = crystals/mother solution ratio

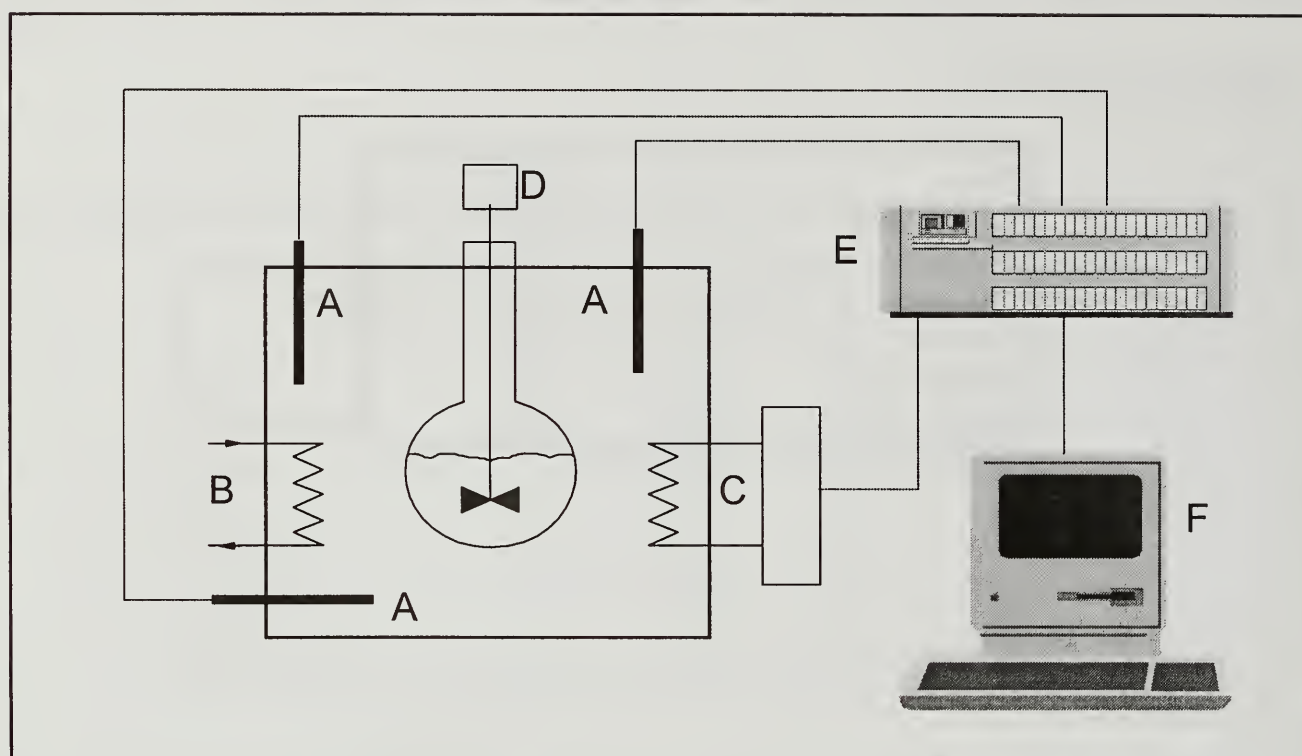


Figure 1. Schematic diagram of the cooling crystallization system: (A) Temperature probe; (B) Cooling device; (C) Heating device; (D) Stirrer; (E) Temperature programmer; (F) Computer.

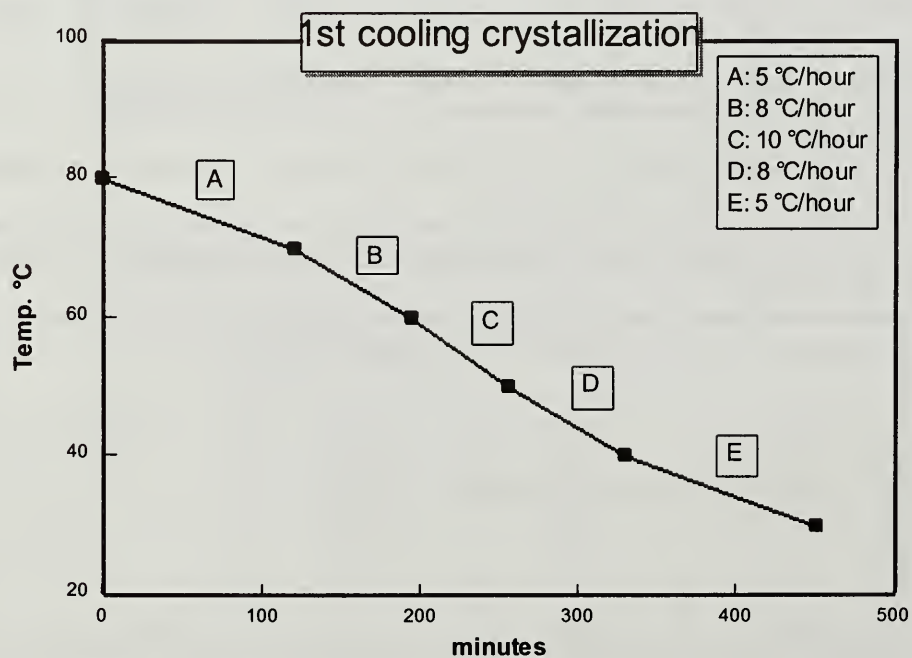


Figure 2. Temperature profile during the 1st crystallization step.

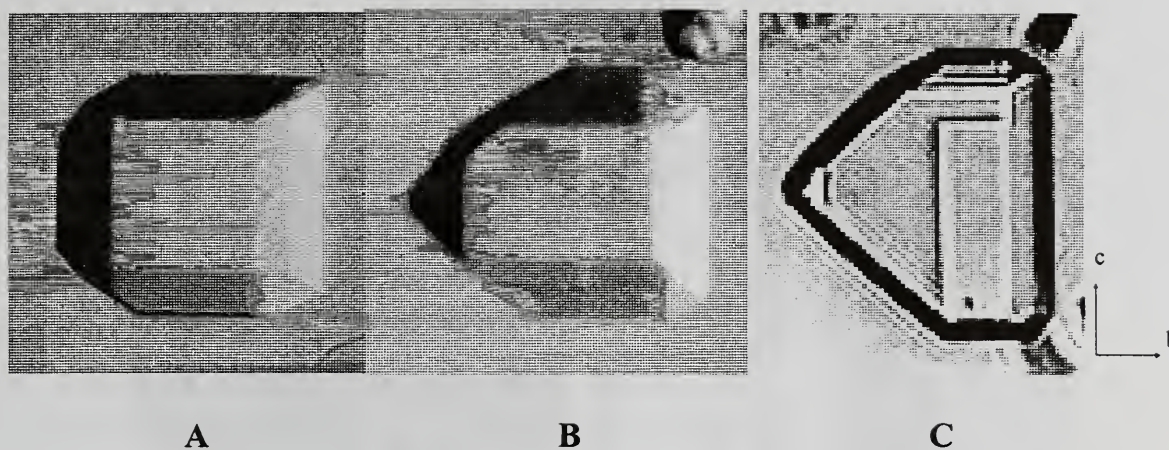


Figure 3. Morphologies of 1st (A), 2nd (B) and 3rd (C) product crystals obtained by cooling crystallization of extract.

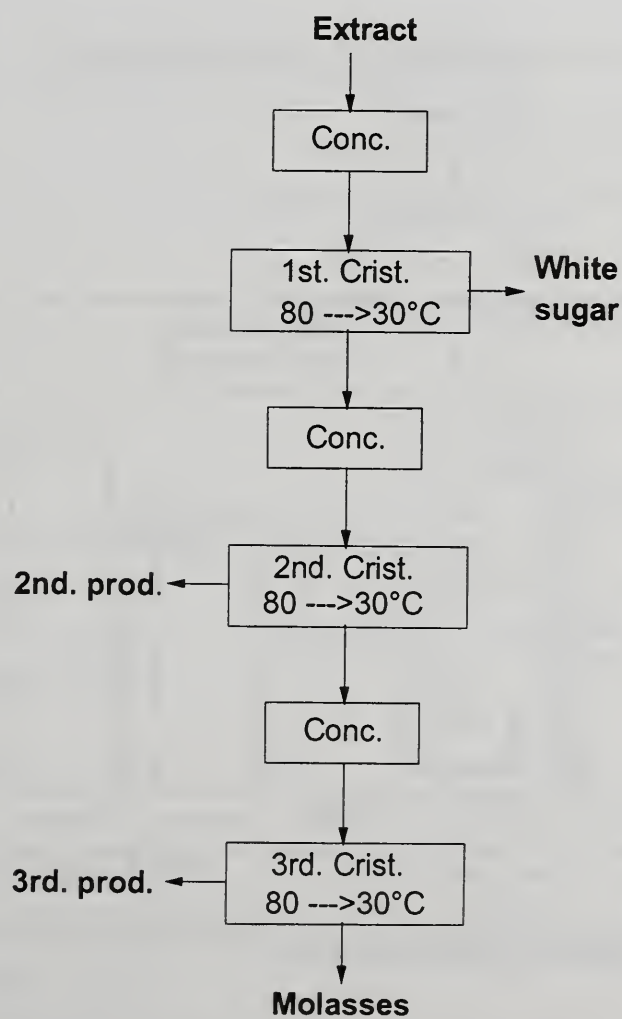


Figure 4. Three-step cooling crystallization scheme for extract from molasses desugarization.

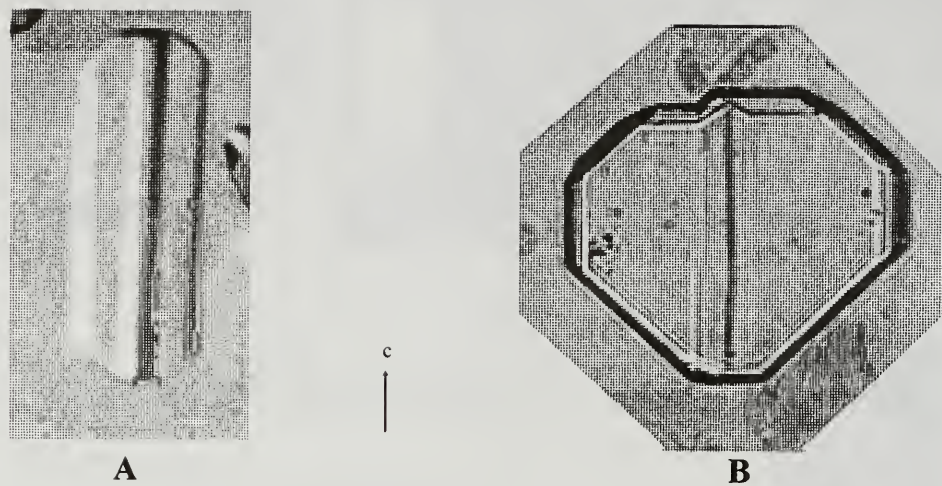


Figure 5. Twins at the beginning (A) and end (B) of their development.

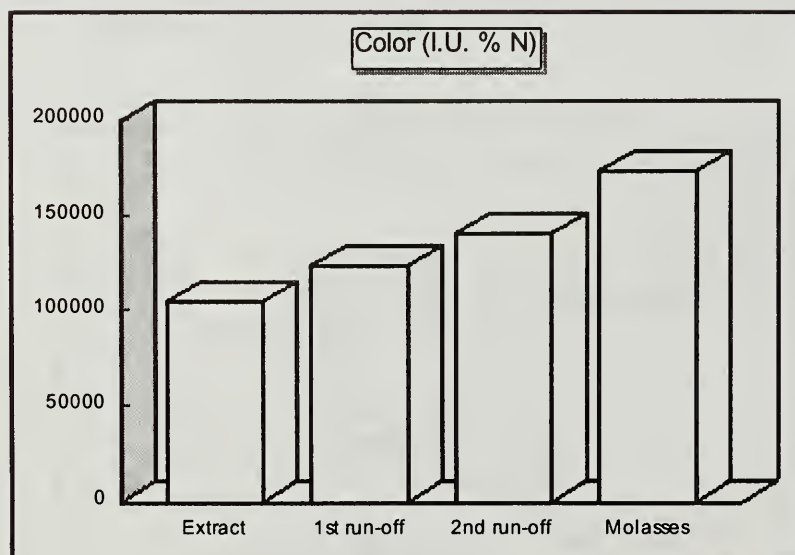


Figure 6. Color rise during the concentration and crystallization steps (N=non-sugar).

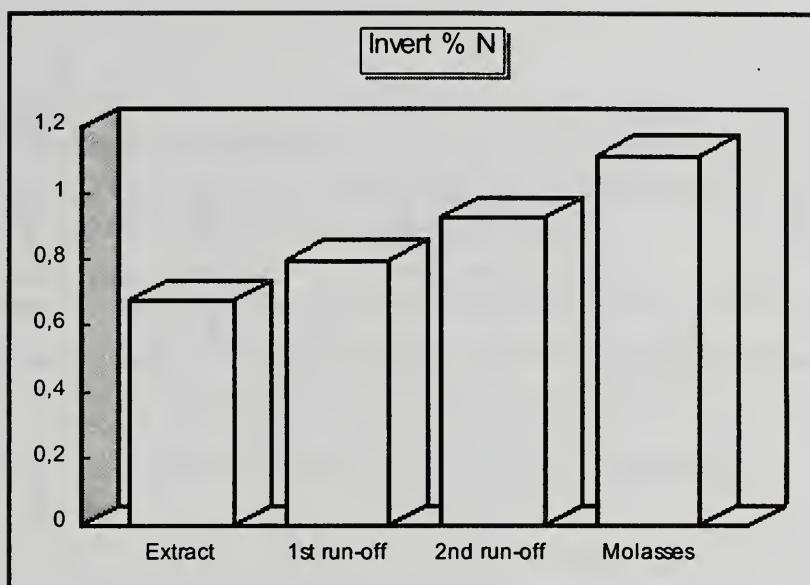


Figure 7. Invert sugar increase during the concentration and crystallization steps (N=non-sugar).

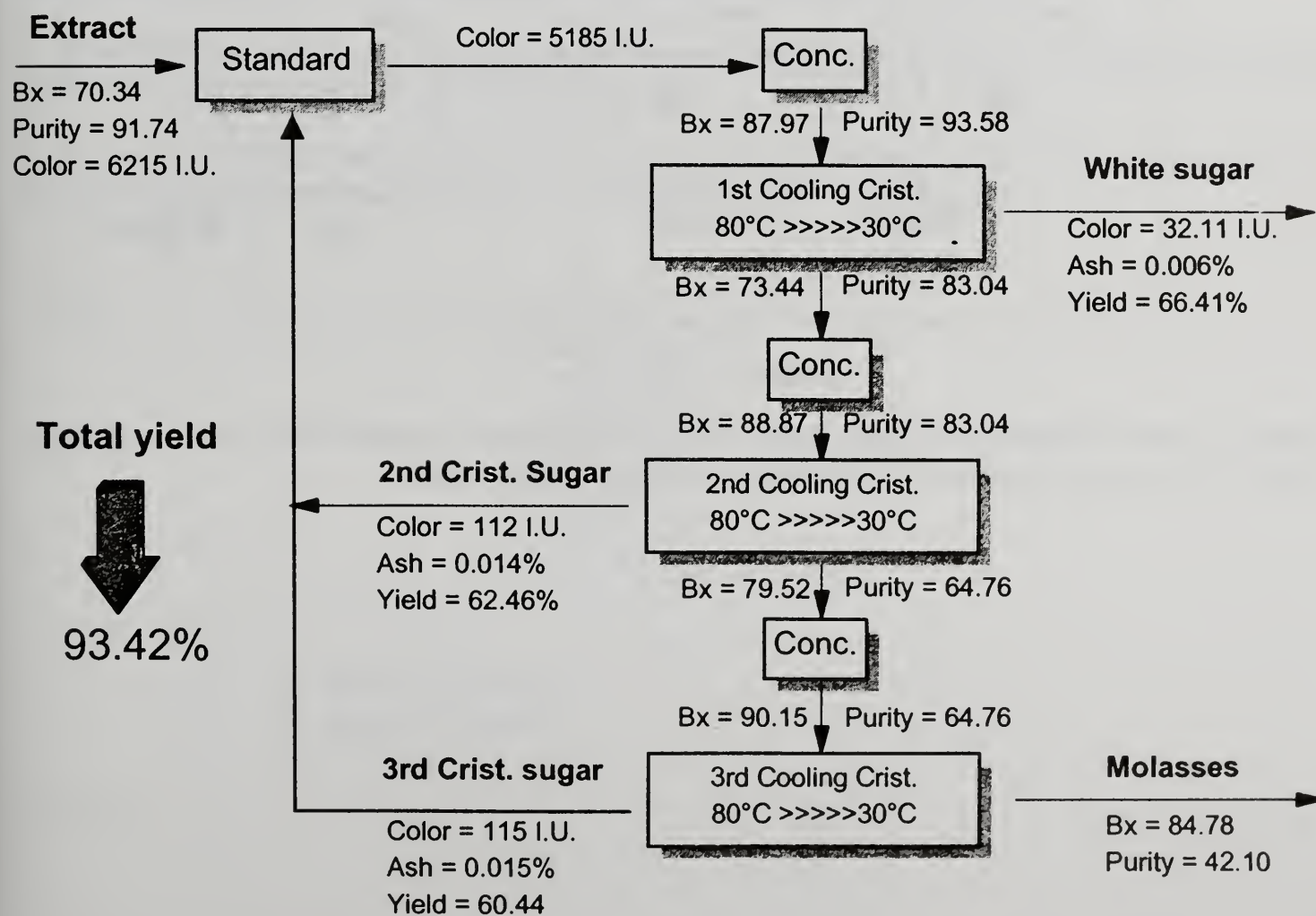


Figure 8. Flow diagram for exhaustive cooling crystallization of extract.

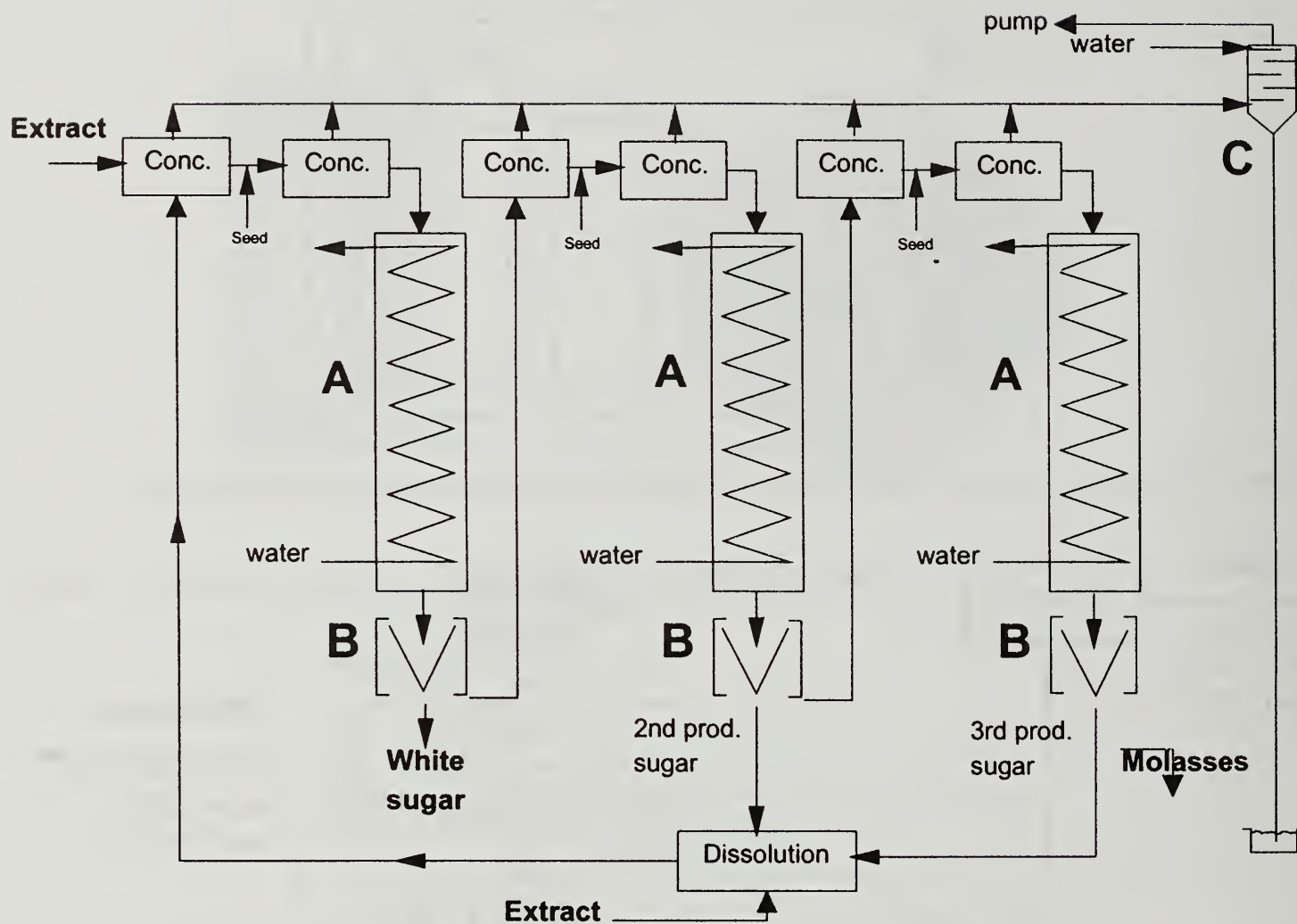


Figure 9. Schematic diagram of a continuous cooling crystallization system for recovery of sucrose from extract: (A) cooling crystallizers; (B) continuous centrifuges; (C) condenser.

DISCUSSION

Question: The combination of techniques was very interesting. I wanted to comment on your D-shaped crystals. They looked like the ones Professor VanHook used to work on. He worked with Pamela Morel du Boil to some extent on these, and Pam found that some oligosaccharides were responsible for the distortion. I wonder if you could comment further about this.

Vaccari: This special morphology, both for the single crystals and twins, is due to the combination effects of raffinose and betaine. As far as single crystals are concerned, we know that raffinose stops the growth on the right pole and promotes the growth only along the left pole (1). In the meantime the betaine dramatically slows down the growth of the q faces of the left pole. The combination effect of the two impurities transforms the traditional morphology of the single crystal as shown in Figure A.

Concerning the twins, it is well known (2) that, in pure solutions, only twins of the first type (p' faces pointing outwards) can be formed. The presence of raffinose (1) transforms the first type twins into the third type (p faces pointing outwards). The additional effect of betaine, which slows down the growing of the q faces, transforms furtherly the third type twins as shown in the sketch of Figure B.

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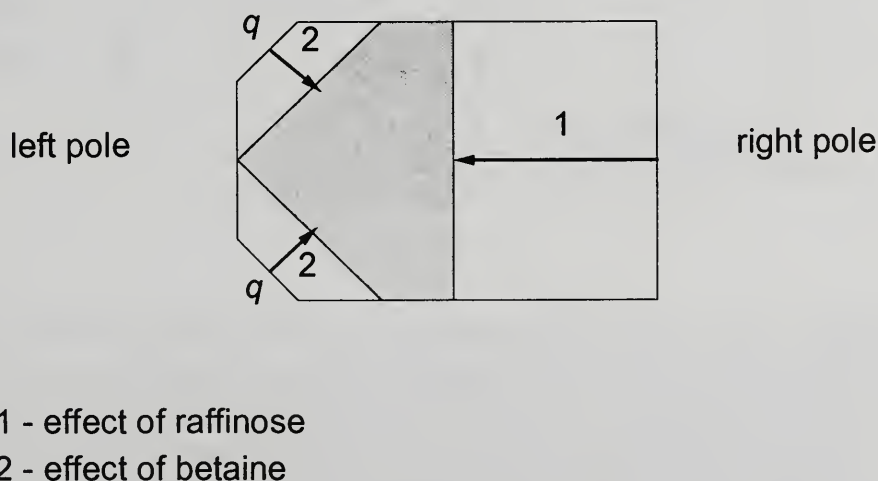


Figure A.

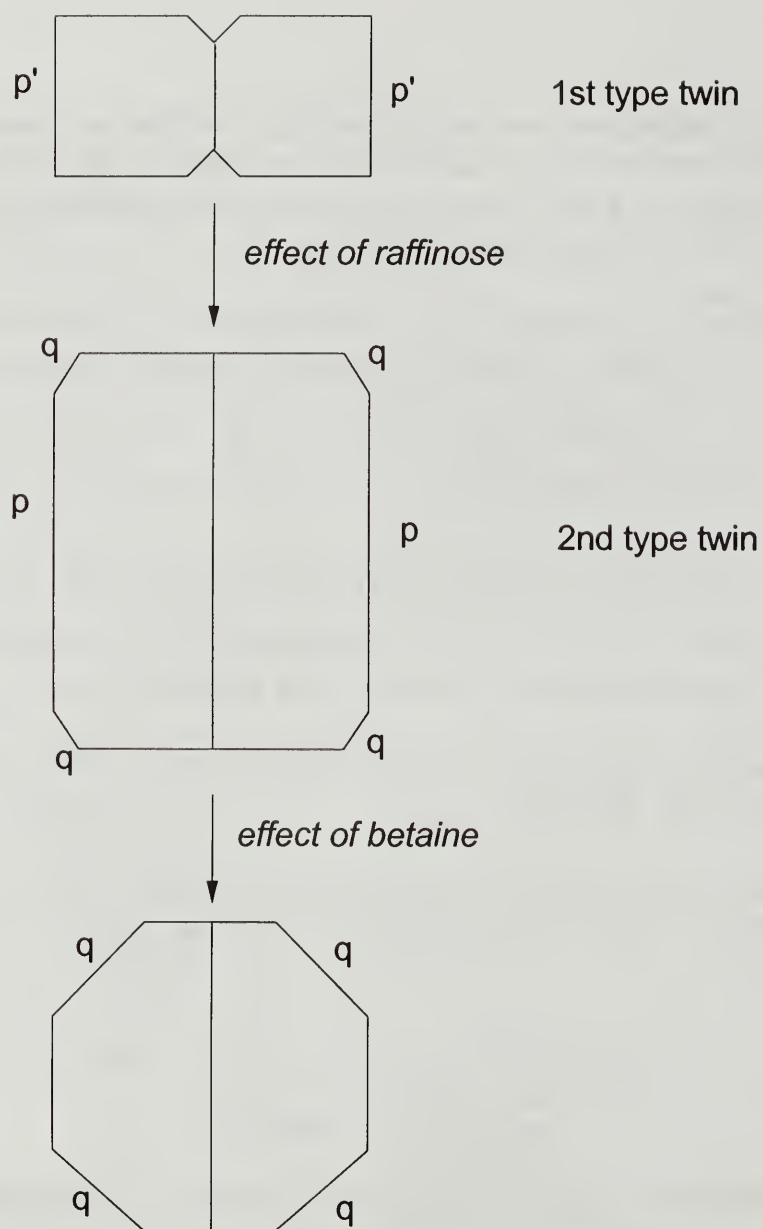


Figure B.

INDUSTRIALLY VIABLE BUILDING BLOCKS FROM KETOSES? STATUS AND PERSPECTIVES

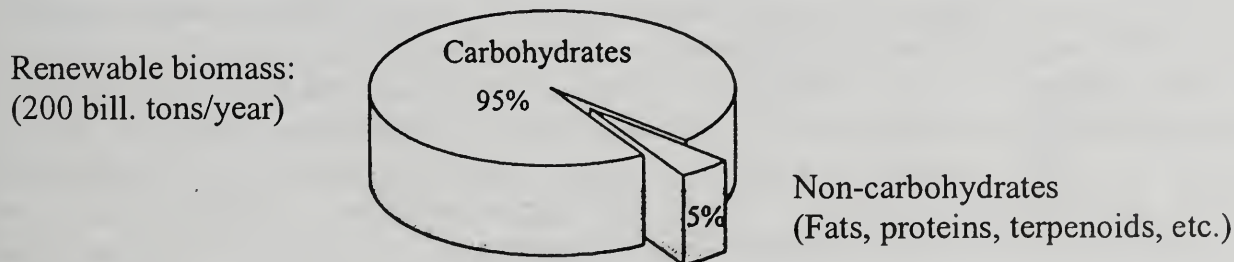
Frieder W. Lichtenthaler, Institute of Organic Chemistry, Darmstadt University of Technology, Darmstadt, Germany

ABSTRACT

Although the ketoses are bulk scale-accessible and inexpensive, i.e. D-fructose, L-sorbose, and D-isomaltulose, their utilization as organic raw materials in the chemical industry is modest -- not surprising in fact, as their chemistry has not developed at a rate comparable with the other common monosaccharides. This account gives an overview of older and very recent efforts to chemically transform these three ketoses into building blocks with industrial application profiles, be it bulk, fine, or specialty chemicals.

INTRODUCTION

Carbohydrates represent roughly 95% of the annually renewable biomass of about 200 billion tons; of these, as of now, only 3% are used by man, the rest decays and recycles along natural pathways.



Although there have been substantial efforts in recent years to step up the chemical industry's use of inexpensive, bulk scale accessible carbohydrates as organic raw materials [1-6], the systematic exploitation of their vast industrial potential is just beginning. There are several reasons for this: First, the use of fossil raw materials is, as of now, still more economic; and second, the process technology for the conversion of petrochemical raw materials into a broad range of products is not only exceedingly well developed but basically different from that required for transforming carbohydrates into products with industrial application profiles. This situation originates from the inherently different chemical structures of the two types of raw materials, of which the essence is already manifested in their structure-based names:

<i>Fossil Resources:</i>	<i>Renewable Resources:</i>
HYDRO-CARBONS	CARBO-HYDRATES
C_nH_{2n+2}	$C_n(H_2O)_n$
oxygen-free, lacking functional groups	over-functionalized with hydroxyl groups

Our fossil resources are *hydrocarbons*, distinctly hydrophobic, oxygen-free and lacking functional groups, whereas the renewables are *carbohydrates*, overfunctionalized with hydroxyl groups and, hence, pronouncedly hydrophilic in nature. Needless to say, the methods required for converting carbohydrates into viable industrial chemicals are diametrically opposed to those prevalent in the petrochemical industry.

As the pressure on our environment increases, and as our fossil resources become depleted, the presently still prevailing economic advantage of petrochemicals is going to change within the time frame of the next 10-20 years, emphasizing the need for developing appropriate process methodology to convert carbohydrates into industrially useful products, be it fibers, packaging materials, fine or bulk chemicals, pharmaceuticals, or simply enantiopure building blocks for organic synthesis.

The bulk of the annually renewable carbohydrate-biomass is polysaccharide, e.g. cellulose, starch, chitin, and inulin, yet their non-food utilization is confined to textile, paper and coating industries, either as such or in the form of simple esters. In terms of their use as basic organic raw materials for the chemical industry, however, the constituent sugars of these polysaccharides -- glucose, fructose (inulin), xylose (xylan) etc., or disaccharide versions thereof -- are considerably more suited for straightforward chemical modifications and, hence, for the elaboration of products with tailor-made industrial applications. In addition, they are *cheap* and *ton scale-accessible* -- a unique situation which becomes particularly evident by the data collected in Table 1. Therein, the bulk quantity prices of the nine least expensive sugars -- all with kg prices well below DM 100, and, hence, ideal starting materials for organic synthesis -- with those of other enantiopure compounds of natural origin and with basic organic chemicals from petrochemical sources. The six least expensive sugars, some sugar alcohols and sugar-derived acids are not only cheaper than any other enantiopure product, such as any of the amino acids or terpenoid natural products, but they compare favorably with such basic organic bulk chemicals as benzaldehyde or aniline. Actually, the four cheapest sugars are in the price range of some of the standard solvents in which organic reactions are usually performed.

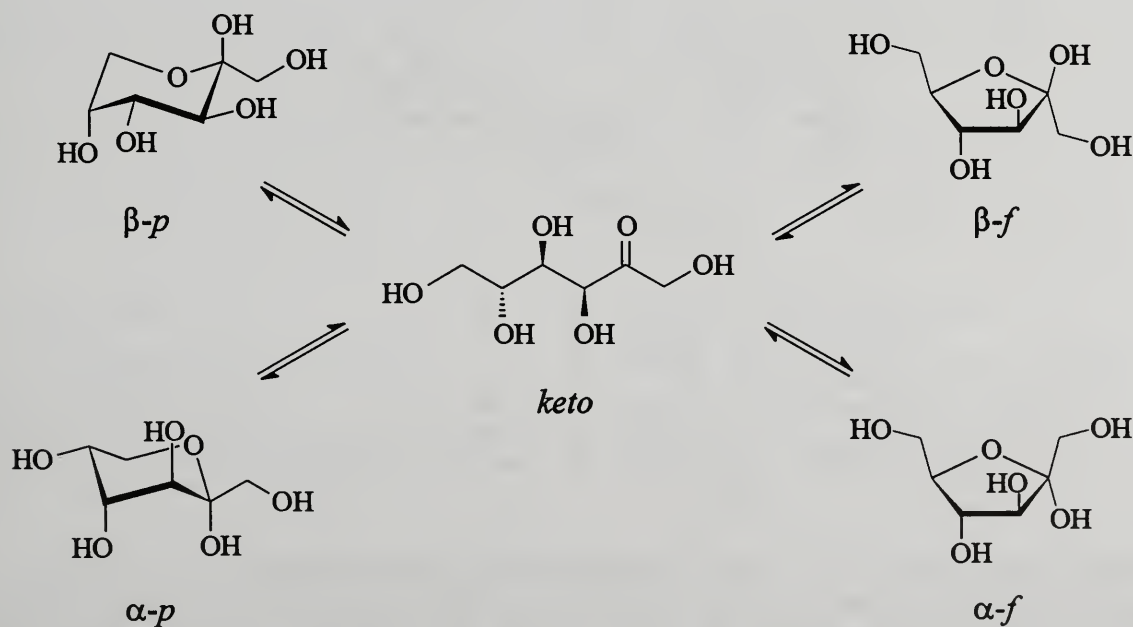
For centuries, sucrose has been the world's most abundantly produced organic compound of low molecular weight, its present annual production being in the 120 million ton range [7]. The present state of its use as an organic raw material has recently been reviewed [6,8,9]. The industrial, non-food valorization of glucose as the most readily accessible aldose is reasonably well developed, comprising its oxidation (\rightarrow D-gluconic acid, a chelating agent and textile printing additive [10]), its reduction (\rightarrow D-sorbitol \rightarrow vitamin C [11]), and

its acid-induced glycosidation with long-chain fatty alcohols to provide alkyl polyglucoside surfactants ("APG's"), presently produced at about a 50,000 t scale per year [12].

In contrast thereto, the non-food utilization of the two comparatively inexpensive, ton-scale accessible ketose monosaccharides, i.e. D-fructose and L-sorbose (cf. Table 1) is particularly modest -- not unexpectedly, as their chemistry is considerably more capricious and less well understood than that of the common aldohexoses. This situation is most strikingly reflected by the fact that the only relevant reviews on the chemistry of D-fructose [13] and L-sorbose [14] appeared as far back as 1952, nearly half a century ago. Accordingly, in terms of the non-food industrial utilization of D-fructose and L-sorbose, as well as of D-isomaltulose, a glucosyl-fructose that recently has become accessible on an industrial scale (*vide infra*), the exploitation of their practically feasible ensuing chemistry appears imperative, i.e. the systematic elaboration of simple and selective "reaction channels" towards products with broad industrial application profiles. It is hoped that this account, reviewing newer developments along these veins, will contribute to the increased use of these renewable ketoses as organic raw materials for the chemical industry.

D-FRUCTOSE

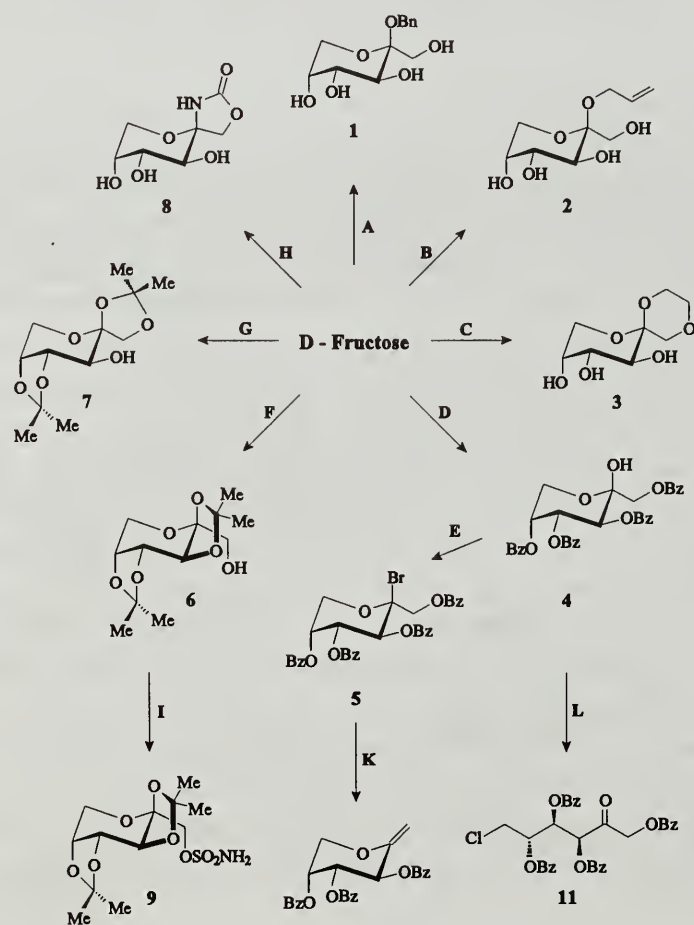
Whilst D-fructose crystallizes in the β -pyranose form as evidenced by X-ray structural analysis [15], in aqueous solution or when dissolved in dimethyl sulfoxide or pyridine, a mixture of the two pyranose and two furanose tautomers is invariably formed, its composition being strongly dependent on the solvent employed and on the temperature [16,17]. In fact, the only tautomer negligible in solution is the acyclic *keto*-form.



Due to this situation, simple derivatizations of D-fructose, such as glycosidations, acylations or alkylations, usually yield product mixtures of at worst, all five tautomeric forms, from which separation of the major component is cumbersome and highly detrimental to the yields obtainable. This particularly applies to acid-

catalyzed glycosidations: exposure of fructose in methanol to an acid (HCl [18], H₂SO₄ [19], or silica-alumina cracking catalysts [20]) leads to complicated mixtures of methyl furanosides and pyranosides which require chromatography for their separation, yields of pure products being in the 20 - 40 % range only. More propitious appears to be the reaction with benzyl alcohol/HCl [21], not because of the higher uniformity of the glycoside mixture generated but because of the fact that the benzyl β -D-fructopyranoside (**1**) can be isolated by crystallization (cf. formula scheme 1). Due to this, **1** has been used as the starting material for the generation of various aminohexuloses [22]. Other, well accessible fructopyranosides are the allyl derivative **2** (49 % [23]), and, most notably, the spiro-glycoside **3**, formed on HCl-promoted glycosidation with 2-chloroethanol and subsequent base-induced spiro-cyclization (74 %) [24].

The most readily accessible acylated fructopyranose is tetrabenzoate **4**, smoothly acquirable from a low temperature benzoylation [25,26] which due to its free anomeric center responds to PCl₅ treatment to give the terminally chlorinated, acyclic *keto*-fructose **11** [26].

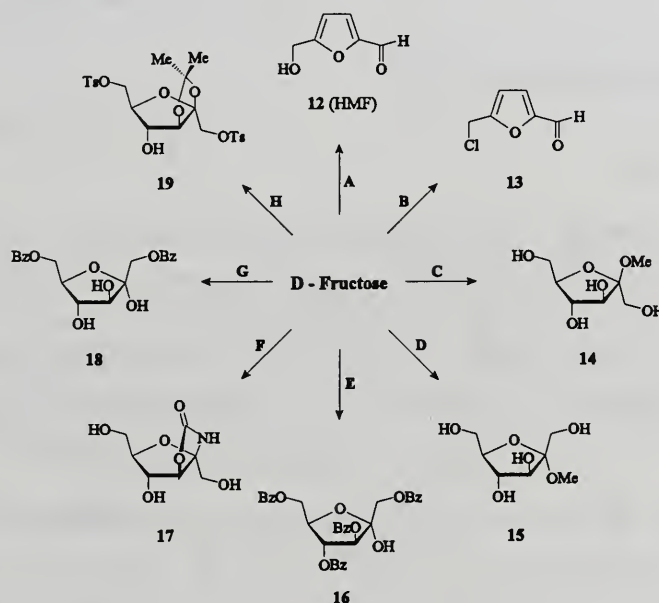


- A BnOH / HCl, 30 % [21]
 B allyl alcohol / AcCl, 49 % [23]
 C 2-chloroethanol / HCl, then NaOMe / MeOH, 74 % [24]
 D BzCl / pyr., -10 °C, 78 % [25,26]
 E HBr, CH₂Cl₂, 0 °C, 90 % [28]

- F acetone / 5 % H₂SO₄, 80 % [30,31]
 G acetone / cat. H₂SO₄, 45 % [32]
 H KOCN, buffer, 31 % [26]
 I ClSO₂NH₂ / Et₃N, 46 % [33]
 K Zn / methylimidazol, 90 % [28]
 L PCl₅, reflux. toluene, 78 % [26]

A versatile key intermediate for the generation of various pyranoid building blocks [27] is the fructopyranosyl bromide **5**, readily accessible from **4** by HBr treatment (90 % [28]), as it can be smoothly converted into *exo*- and *endo*-fructal esters, e.g. **10** [28], or the respective hydroxyfructals [27,29]. Of similar preparative importance are the two diacetone-fructopyranoses **6** [30,31] and **7** [32], as only one of the five OH groups is unblocked and, hence, available for versatile further modifications; sulfamoylation of the primary OH group in **6**, for example, leads to topiramate **9**, a novel drug with high antiepileptic efficacy [33]. Similar synthetic as well as conceivable pharmaceutical potential may apply to the cyclic carbamate **8**, smoothly generated on reaction of fructose with potassium cyanate [26].

These "entry reactions" from fructose to simple derivatives fixed in pyranoid form are complemented by an equally multifaceted array of readily accessible furanoid compounds, i.e. products **12-19**, of which structures and mode of synthesis are compiled in the following scheme:



A H^+ , 90 % [35,36]

B HCl / toluene, 60-85 °C, 65 % [37]

C MeOH / HCl , 33 % [18]

D MeOH /silica-alumina catalyst, 30 % [20]

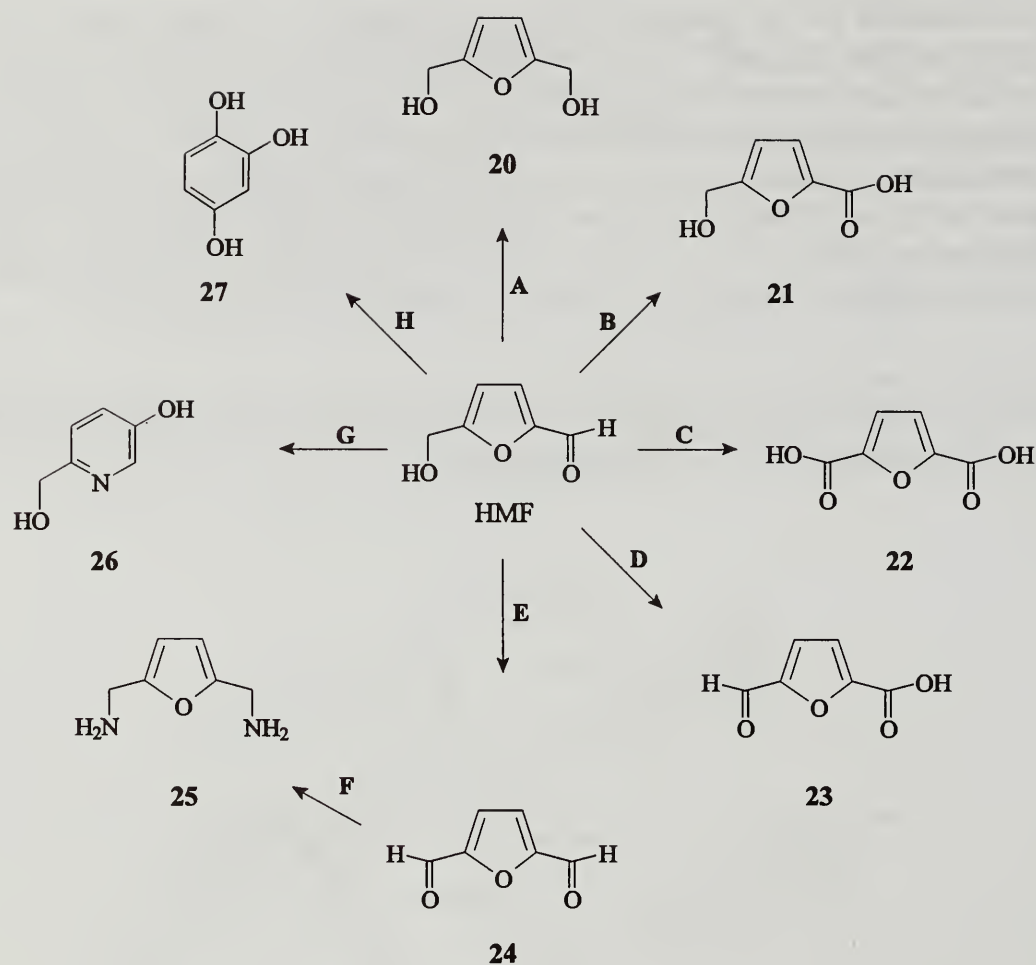
E BzCl / pyr., 60 °C, 60 % [38]

F KOCN / buffer, 32 % [26]

G HCN / NH_3 , then BzCl / pyr., finally Ag_2CO_3 / EtOAc / reflux, 60 % [39]

H TsCl / pyr., then Me_2CO / H^+ , 20 % [40]

Of the simple furanoid products derived from fructose, by far the highest industrial potential is for hydroxymethylfurfural ("HMF", **12**), which has been termed one of the few "petrochemicals" readily accessible from renewable resources [34] and is a key substance between carbohydrate chemistry and mineral oil-based industrial organic chemistry [35]. It is readily produced by acid-induced elimination of 3 moles of water [36], and even a pilot plant size process has been elaborated [35].

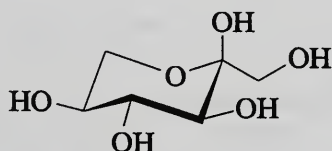


- | | |
|---|--|
| A Pt,C / H ₂ , quant. ^[41] | F NH ₂ OH, then Ni / H ₂ , 33 % ^[44] |
| B Pb-Pt / C, O ₂ , NaOH, 70 % ^[42] | G Ni / H ₂ / NH ₃ , then Ac ₂ O, finally |
| C Pt / C, O ₂ , pH 7, 91 % ^[43] | electrolysis, 49 % ^[44] |
| D Pt / Al ₂ O ₃ / pH 9, 75 % ^[44] | H H ₂ O / 330 °C / Δp ^[47] |
| E BaMnO ₄ , 93 % ^[44] | |
| Ca(OCl) ₂ / TEMPO, 81 % ^[45] | |

As of now, however, HMF is not produced on an industrial scale, despite of the fact, that its conversion into various ensuing products are well worked out. Compounds **20-27**, for example, have great potential as basic industrial intermediates in the manufacture of polyamides, most notably the dicarboxylic acid **22** and the diamine **25**, as they are conceivable replacements for terephthalic acid and hexamethylene diamine, respectively. Indeed, various polyamides have been prepared utilizing **22** as the dicarboxylic acid component and aliphatic as well as aromatic diamines [48-50], yet despite interesting properties such as films, fibres and/or liquid crystals, they are industrially not utilized at present, obviously because polyamides based on petrochemical raw materials are still more economical [51].

L-SORBOSE

L-Sorbose, the 5-epimer of D-fructose, is the most readily, large-scale available L-sugar (cf. Table 1) due to its technical production from D-sorbitol in the vitamin C fabrication process [11]. However, its non-food utilization has been exceedingly poor despite the fact that the α -pyranoid tautomeric form is highly favored in solution (87-98 %) in the temperature range of 27-85 °C [16], on account of which the "entry reactions" to pyranoid derivatives are proceeding in a more uniform manner and allow better yields than in the fructose case.

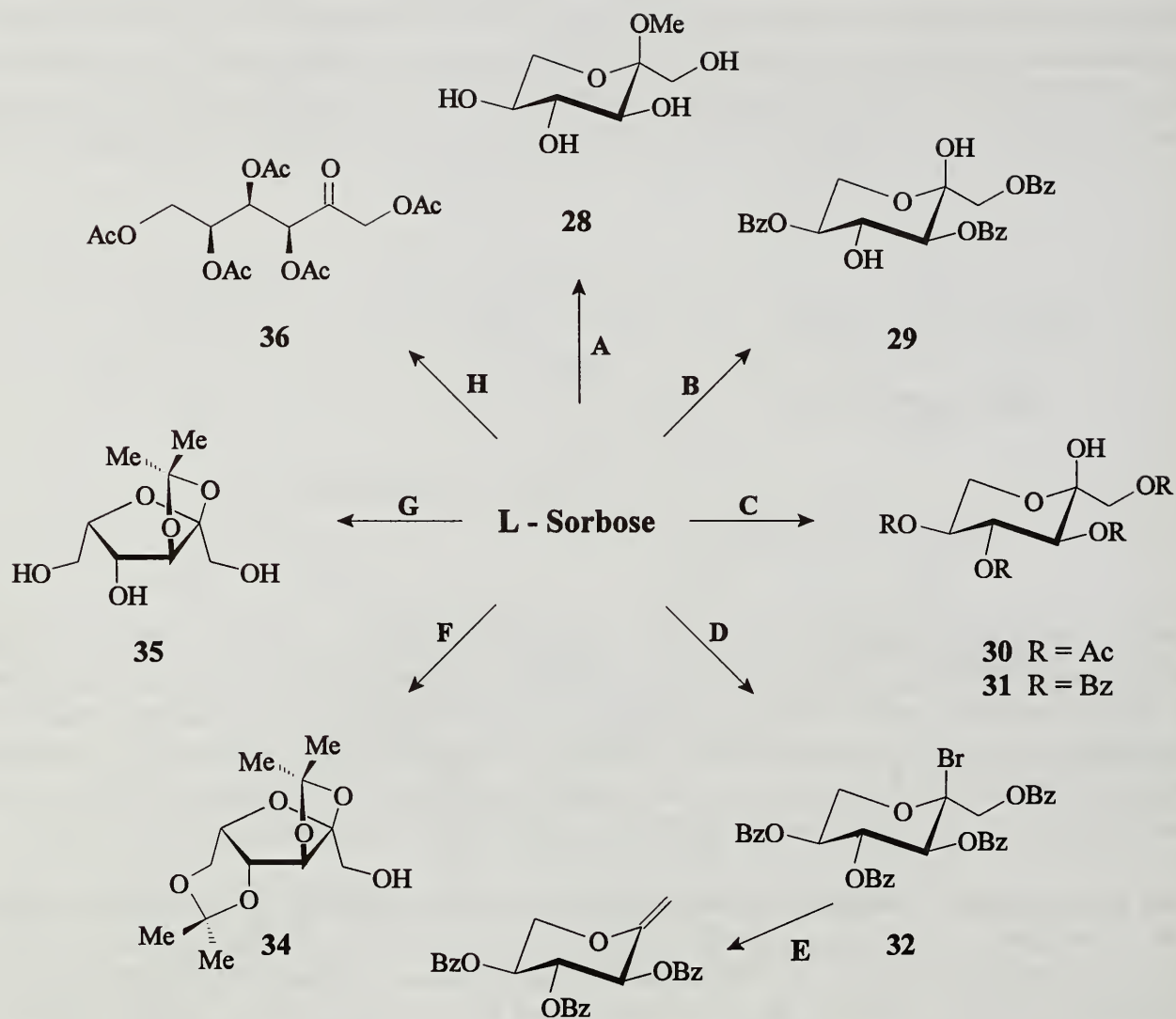


α -L-sorbopyranose

Accordingly, Fischer-type glycosidation with methanol/HCl affords a 95 % yield of the methyl α -L-sorbopyranoside **28** [18]. Low temperature benzylation proceeds selectively to the 1,3,5-tribenzoate **29** [52], whilst acylation under standard conditions at low temperature yet prolonged reaction times smoothly affords the pyranoid tetraacetate **30** [53] and tetrabenzoate **31** [54], respectively. If benzylation is followed by exposure to hydrogen bromide, the sorbopyranosyl bromide **32** is obtained [54], which serves as an ideal precursor for the generation of the highly versatile *exo*-sorbal building block **33** [28].

Of the two well accessible L-sorbose derivatives fixed in furanoid form, the 2,3:4,6-di-*O*-isopropylidene acetal **34** is available in ton-scale due to being a key intermediate in the Vitamin C fabrication process [11,55], whilst the acquisition of the mono-*O*-isopropylidene compound **35**, primary product of the acid-catalyzed acetonation towards **34**, is less optimized [56]. Of the various acyclic *keto*-L-sorbose derivatives known, the pentaacetate **36**, smoothly formed on zinc chloride-promoted acetylation [57], appears to be the most easily preparable.

The array of well accessible pyranoid, furanoid and open-chain derivatives of L-sorbose is very similar to that derivable from D-fructose -- not unexpectedly, as the two hexoses are 5-epimers only. The major differences in their preparative utilities appears to lie in the structures of their isopropylidene acetals, D-fructose invariably yielding pyranoid diacetanides with either 1-OH and 3-OH free (**6** and **7**, resp.), whilst L-sorbose only elaborates the furanoid mono- (**35**) or di-acetonide (**34**) with the hydroxyl groups at C-1, C-3 and C-6, or at C-1 unprotected. The use of L-sorbose derivatives, of course, will also be advantageous if the configuration of a given enantiopure target molecule matches better with its array of chiral centers than with those of D-fructose.



A MeOH / HCl, 95 % ^[18]

B BzCl / pyr., -10 °C, 43 % ^[52]

C Ac₂O / pyr., -10 °C, 65 % ^[53]

BzCl / pyr., -10 °C, 71 % ^[54]

D BzCl / pyr., -10 °C, then HBr, 68 % ^[54]

33

E Zn / methylimidazol, 80 % ^[28]

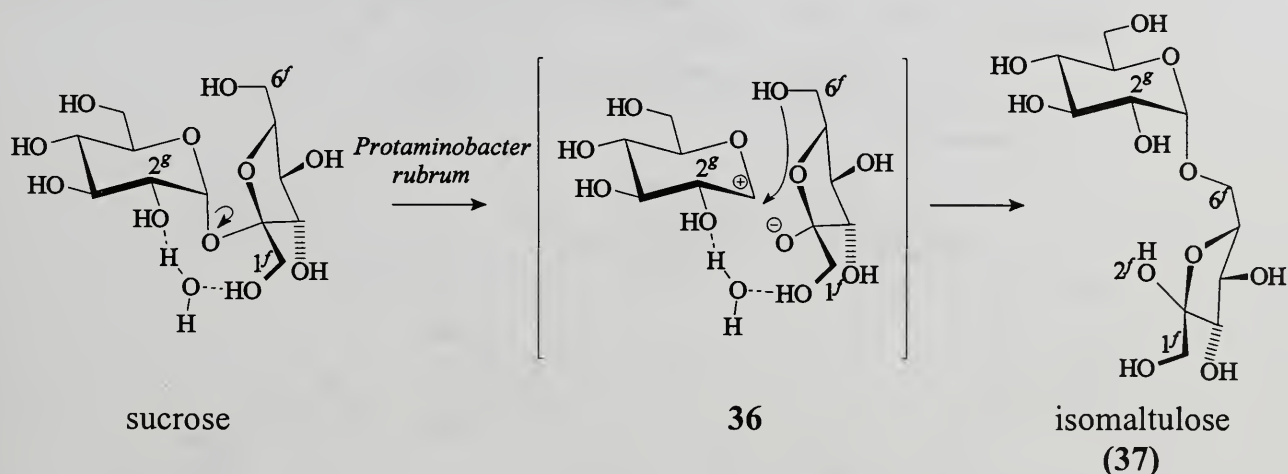
F Me₂CO / H₂SO₄, 4 °C, >90 % ^[55]

G Me₂CO / H₂SO₄, 40 % ^[56]

H ZnCl₂ / Ac₂O, 70 % ^[57]

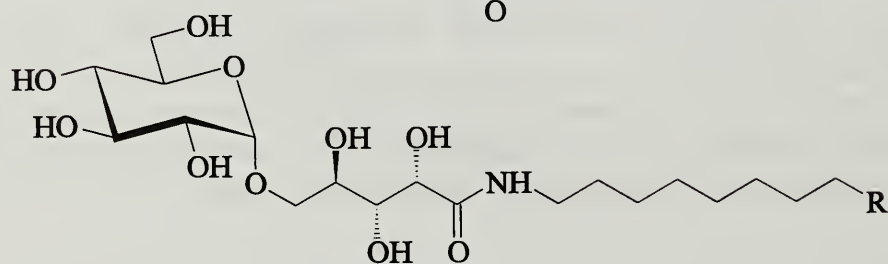
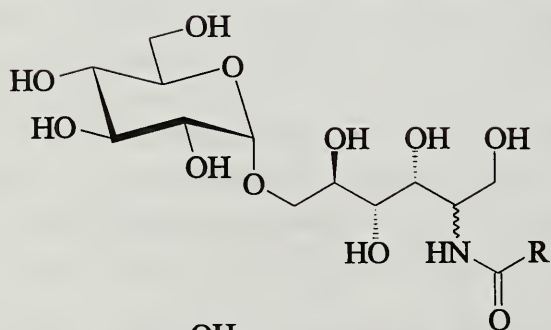
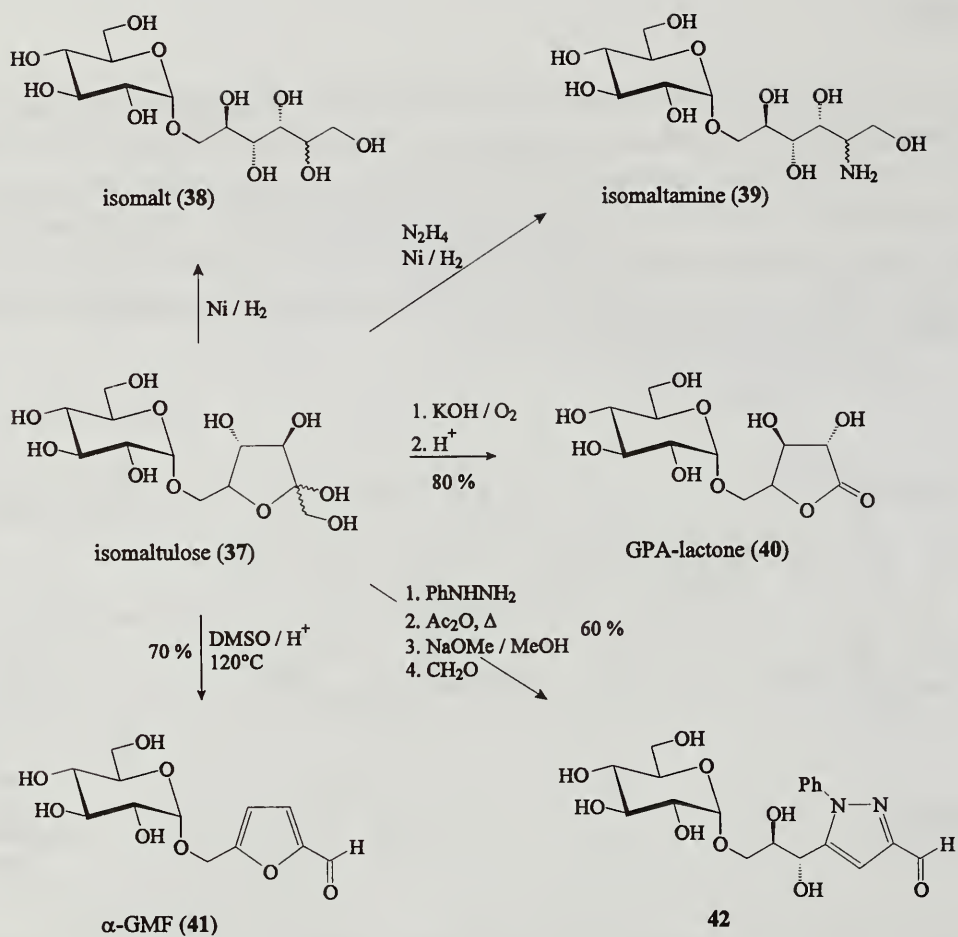
D-ISOMALTULOSE

This disaccharide, a 6-*O*-glucosyl-D-fructose (37), has also been designated "palatinose" due to its first isolation from molasses [58] in the Südzucker Research Laboratories at Offstein in the Palatinate section of central Germany. It is produced by Südzucker on a 30,000 ton per year scale presently by causing sucrose to undergo a *Protaminobacter rubrum*-induced glucosyl-shift [59,60], a transformation that most likely proceeds via a closed-shell transition state of type 36 [8], as due to the water bridge between 2^g-O and 1^f-OH [61] both monosaccharide portions conceivably exist as a glucosyl-cation/fructosyl-anion ion pair, in which the 6^f-OH is sterically close to the glucosyl-C-1:



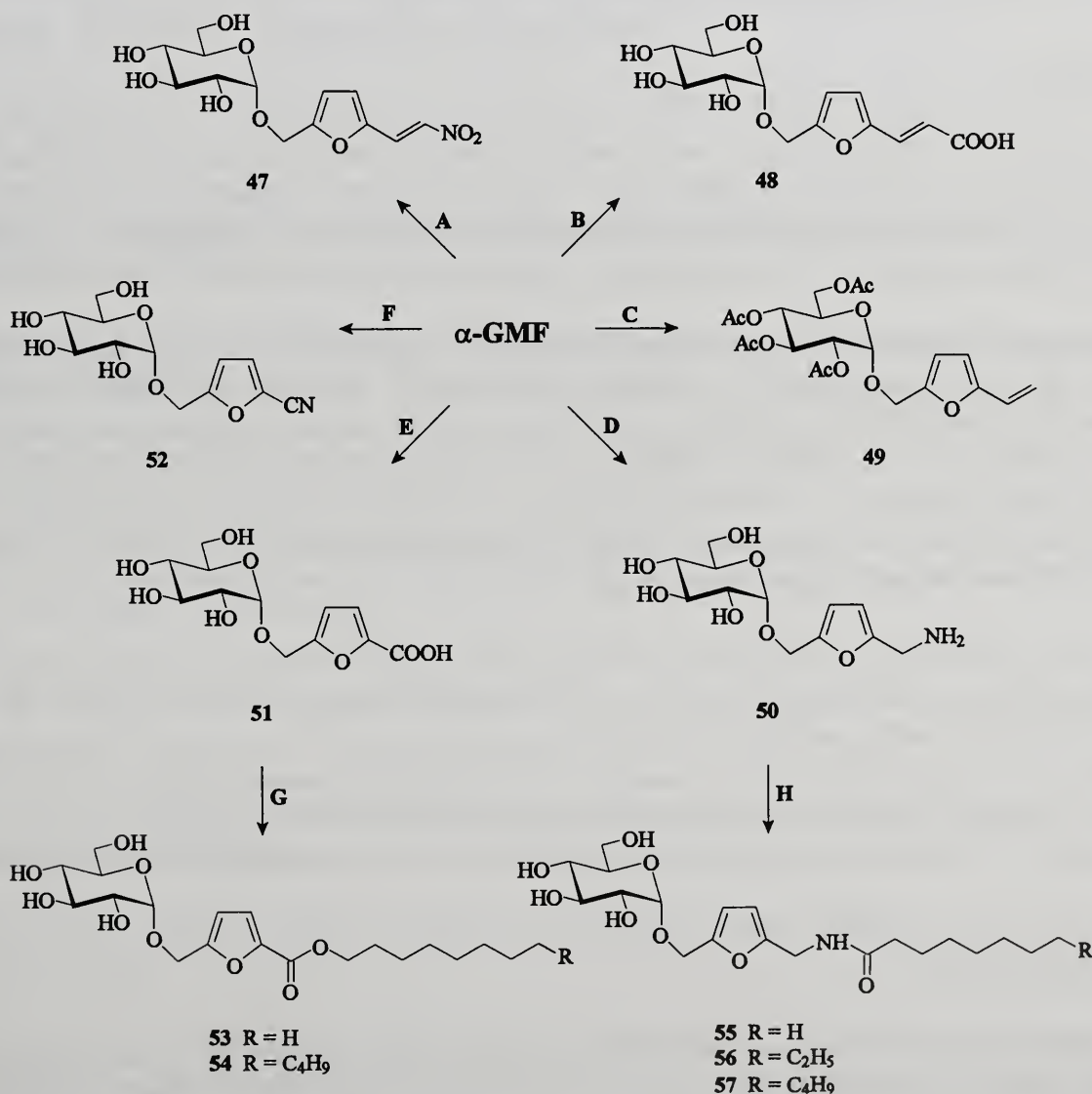
Isomaltulose crystallizes well as a dihydrate in its α-furanoid tautomeric form [62]. In solution, the number of tautomers are reduced to half (as compared to D-fructose) because due to the non-availability of the 6-OH in the fructose portion only the two furanoid tautomers are prevailing, of which the β-*f*-form usually preponderates by about 3:1 [63]. In any of its derivatives, the fructose portion will either be furanoid or acyclic. Thus, catalytic hydrogenation yields a 1:1 mixture of the 6-*O*-glucosylated D-mannitol and D-glucitol (38) [59,60], which has been named "isomalt"[®] and is used as a low caloric sweetener with essentially the same taste profile as sucrose [59,60]. Non-food applications result from its reductive amination with hydrazine on a nickel catalyst which smoothly generates a 1:1-mixture of glucosyl-α(1→6)-2-amino-2-deoxy-D-glucitol and the respective D-mannitol isomer [64], appropriately termed isomaltamine (39) in analogy to isomalt[®]. Air oxidation of isomaltulose in strongly alkaline solution (KOH) gives the potassium salt of the next lower aldonic acid, i.e. glucosyl-α(1→5)-D-arabinonic acid ("GPA"), isolable as the potassium salt or, after neutralization, as the GPA-lactone 40 in high yields each [65].

Another industrially relevant reaction of isomaltulose comprises its ready conversion into 5-glucosyloxymethyl-furfural ("α-GMF", 41) by acidic dehydration of the fructose portion under conditions that retain the intersaccharidic linkage [66]. This process, i.e. 37 → 41 can also be performed in a continuous flow reactor [67]. A preparatively satisfactory route to hydrophilically functionalized pyrazoles of type 42 was also developed recently, requiring only three steps and simple reagents [68].



Various products with industrial application profiles have been prepared from the modified isomaltulose building blocks **39-42**. Isomaltamine **39**, for example, as a pronouncedly hydrophilic amine of a disaccharide alcohol, it is a versatile intermediate for further derivatization, e.g. with fatty acid halides to non-ionic, biologically degradable detergents of type **43** [69], or with methacrylic acid derivatives to provide polymerizable acrylamido-disaccharides of type **44** [64]. Amidation of GPA-lactone **40** with long-chain amines, e.g. the C₈- and C₁₂- "fat amines", provided the GPA-amides **45** and **46**, which not only exhibit promising detergent profiles, but surprising liquid crystalline properties, such as S_{Ad}-phases over a broad temperature range [70]. Thereby, the X-ray-derived length of molecules are distinctly larger than those calculated for the extended, non-associated species, which suggests the formation of dimers in which sections of the two molecules are partially overlapped.

Ensuing Chemistry of α -GMF [66]



- | | |
|--|--|
| A $\text{CH}_3\text{NO}_2 / \text{NaOH}$ | E $\text{NaClO}_2 / \text{H}_2\text{O}$ |
| B $\text{CH}_2(\text{COOH})_2 / \text{quinoline} / 170\text{ }^\circ\text{C}$ | F $\text{NH}_2\text{OH} / \text{HCl} / 110\text{ }^\circ\text{C}$ |
| C $\text{Ac}_2\text{O}^\oplus \text{CH}_2\text{Br}_2 / \text{Zn} / \text{TiCl}_4$ | G fat-alcohol / DCC |
| D $\text{Ni} / \text{H}_2 \text{ in MeOH} / \text{NH}_3$ | H acyl chloride |

As a glucosylated HMF, α -GMF (**41**) provides a rich ensuing chemistry towards products with broad application profiles [66]: Aldol-type condensations deliver derivatives with polymerizable double bonds (routes A and B), most notably the acrylic acid **48** and methylenation product **49**, that are expected to yield novel, hydrophilic polymers with interesting performance profiles; reductive amination smoothly generates the α -GMF-amine **50**, which by *N*-acylation with fatty acids affords compounds of type **55-57**, i.e. non-ionic surface-active agents, in which the hydrophobic fat-alkyl residue and the hydrophilic glucose part are separated by a quasi-aromatic spacer; similar application potential pertains to the α -GMF-carboxylic acid **51** and its esters with long-chain alcohols [66].

CONCLUSIONS

Despite the various new "entry reactions" and "reaction channels" advanced here for the three bulk-scale accessible ketoses, their potential as organic raw materials for the elaboration of industrially useful chemicals is far from being fully exploited. Numerous further reactions are conceivable through application of modern methodologies and wait for elaboration -- a situation that holds globally for all carbohydrates.

This, unambiguously, points towards the need for broad-scale, practically-oriented basic research to be performed on the entire spectrum of promising applications, in order to decisively improve the competitiveness of accessible low molecular-weight carbohydrates as basic organic raw materials. A precondition for auspicious advances towards this end is, however, that the chemical industry becomes actively engaged in the basic research to be performed and gives up its present wait-and-see attitude: wait for industrially interesting results elaborated in academic institutions, and only then see, how they can be exploited towards lucratively marketable products.

In striving for the replacement of fossil raw materials by renewable resources, it would be an unrealistic strategy to try to generate from carbohydrates, i.e. 95 % of the biomass annually regrowing, the very same basic chemicals that are easily produced from petrochemical sources. The objective emerging from the present scenario is another one, the only reasonable one, in fact: development of products from renewable resources with analogous industrial application profiles and with as little alteration of their structural framework as possible. Only then economically sound alternatives to petrochemicals will become available.

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Table 1. Availability and prices of low molecular weight carbohydrates compared to petrochemically derived basic organic chemicals and solvents.

		World Production* (t / year)	Price** (DM / kg)	Source (Supplier)
<i>Sugars</i>	Sucrose	123,000,000	0.75	World Market
	D-Glucose	5,000,000	1.15	Cerestar
	D-Lactose	295,000	1.20	Borculo Whey
	D-Fructose	60,000	2.00	Südzucker
	D-Maltose	3,000	5.00	Cerestar
	D-Isomaltulose	30,000	5.00	Südzucker
	D-Xylose	16,000	12.00	Xyrofin
	L-Sorbose	25,000	35.00	Merck
	D-Galactose	?	85.00	Fluka
<i>Sugar Alcohols</i>	D-Sorbitol	650,000	2.00	Merck
	D-Mannitol	20,000	6.00	Cerestar
	D-Xylitol	15,000	12.00	Xyrofin
<i>Sugar-derived</i>	D-Gluconic Acid	60,000	7.00	Fluka
<i>Acids</i>	L-Tartaric Acid	?	10.00	Merck
	L-Ascorbic Acid	60,000	10.00	Merck
<i>Amino Acids</i>	L-Glutamic Acid	250,000	15.00	
	L-Lysine	40,000	20.00	
<i>Industrial Organic</i>	Acetaldehyde	900,000 [†]	1.00	
<i>Chemicals</i>	Aniline	1,320,000	1.50	
	Benzaldehyde	50,000	3.60	
<i>Solvents</i>	Methanol	26,500,000 [†]	0.30	
	Toluene	6,500,000 [†]	0.50	
	Acetone	3,200,000	0.75	

* Reasonably exact data are available for the world production of sucrose [7]; all other data are average values based on more or less comprehensive estimations from producers and/or suppliers.

** Prices given are those attainable in mid-1997 on the world market for bulk delivery (ton range), or in the EU after allowing for EU refunds for industrial utilization of the sugars listed.

[†] Data from "The Chemical Industry in 1994", UN Annual Review, New York/Geneva 1995.

SUGAR DEGRADATION AND COLOUR FORMATION

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ABSTRACT

In beet sugar manufacture there are two chemical phenomena which should be controlled as tightly as possible.

First, sugar degradation results in the loss of sugar and the concomitant formation of (reactive) invert sugar. Particularly extraction and evaporation are considered to be important process steps in which a small, but significant, part of the sugar can be degraded by acid hydrolysis.

The colour of the juices finds its origin in juice purification, and will depend on both the quality of the processed beets and the way the diffusion process and juice purification are carried out. In addition, a further colour increase is generally observed in the evaporation station and the subsequent crystallization steps. With respect to product quality a too high colour formation is undesirable and, therefore, should be prevented.

The latest insights at CSM concerning both sugar degradation and colour formation in its beet sugar factories will be presented. Emphasis will be given to the sources and process parameters which may be responsible for colour formation. A model laboratory study is in progress in order to determine which juice components and/or process parameters have to be considered as mainly responsible for the observed juice colour.

INTRODUCTION

Sugar degradation and colour formation are two matters of concern in sugar manufacture. Sugar degradation results in the loss of sugar and so determines the final yield of obtainable white sugar. Colour formation makes the production of 'white' sugar after crystallization more difficult and at least an increased wash in the centrifugals will be needed in order to meet specifications for sugar colour and colour in solution (12). It can be concluded that these chemical phenomena are undesirable and, therefore, should be controlled as tightly as possible.

Both phenomena have long been major issues in sugar technology, as indicated by several review articles published in the literature on this subject (7, 9, 15) and the fact that they were the main topics of the general assembly of the 'Commission Internationale Technique de Sucrerie', held in Paris (1964) and in Ferrara (1987), respectively. As a consequence, most of the parameters which contribute to sugar degradation and colour formation in process are known, but still more specific insights are required in order to be able to control these chemical reactions properly.

The quality of the processed beets will have a considerable influence on both phenomena. The parameters usually applied for a description of the internal beet quality are potassium, sodium and α -amino nitrogen; sometimes the invert sugar content of the beets is considered also. In addition to this, external factors like the percentage of green material and beet top entering the process (9) will influence the processability of the beets, particularly with respect to colour formation. The so-called technological value of sugar beets is often incorporated into the payment system from the sugar manufacturer to the farmers. The way the beets are processed may have an even larger impact on sugar degradation and colour formation than beet quality.

The variation of beet quality between different countries, campaigns and just during one campaign period as well as the differences in sugar beet processing among factories make it very difficult to translate research literature data of others to a local situation. For that reason we decided to gather more detailed information on the origin and extent of sugar degradation and colour formation in our CSM factories. A further optimization of the process is the aim of such an investigation, as the outcome should allow us to control more properly those parameters which appear to be of major importance.

This paper will give an outline of the research we recently carried out on this subject. First we have recorded the actual situation in our factories, with emphasis on diffusion, juice purification and evaporation. Some practical examples will be described. Additionally model laboratory experiments have been carried out in order to determine those parameters responsible for sugar degradation and colour formation in process and to which extent they influence these chemical reactions.

METHODS AND MATERIALS

Laboratory juice purification

On a laboratory scale model juice purification experiments have been carried out, including preliming, cold and hot main liming, first carbonatation and filtration.

In case a synthetic raw juice is used, a choice is made of a combination of the next ingredients which are dissolved in 3 litres demineralized water:

- 450g sugar (present in all experiments)
a mixture of organic acids and salts (totally about 21g), which are commonly found in raw juice; i.e. potassium, sodium, magnesium salts of chloride, nitrate, phosphate, sulfate, sulfite, malate, oxalate and citrate (present in all experiments)
- 3.18g glucose and 1.35g fructose (or the double amount of both)
- 1.0 g γ -amino butyric acid or 1.0g glutamine
- 3g of pressed beet pulp
- 3g of green beet material

- a pre-oxidized solution of L-dopa (dihydroxyphenylalanine); further details on the preparation of this solution are mentioned in 'Results and discussion'.

The pH of the model raw juices is about 5.8. In the various experiments carried out, the composition of the synthetic raw juice has been varied in order to determine the individual effect of the different parameters on colour formation.

The procedure of the laboratory juice purification is as follows:

1. In a jacketed 3 litre glass vessel (synthetic) raw juice is heated to 55°C.
2. At the preliming stage milk of lime (150g/l Ca(OH)_2 in demineralized water) is added progressively during 30 minutes, with 3 minutes intervals, to a final alkalinity of ~190mg/100ml CaO.
3. After 15 minutes residence time at 55°C an excess milk of lime is added to an alkalinity of ~1000mg/100ml; the cold main liming lasts 15 minutes.
4. The juice is pumped to a second vessel in which it is heated within 20 minutes to 85°C; the hot main liming at this temperature is completed after 10 minutes. A sample is taken, filtered and cooled, for further analysis.
5. During the first carbonatation carbon dioxide from a gas cylinder is added to the limed juice. The pH is measured in the circulation juice and the carbonatation is finished if the actual hot pH reaches 10.0, which equals pH~11.0 at ambient temperature. A sample is taken, filtered and cooled, for further analysis.

The samples taken after the main liming and first carbonatation are generally analyzed for pH, alkalinity, brix, and colour at 420nm. In some cases special analyses are carried out, e.g. chromatographic analysis of organic acids and anion composition (1, 4) invert sugar content (HPAEC method similar to the provisional ICUMSA method as described in 1997 by Mr. K. Schaffler, referee of Subject 8, 'Chromatographic Techniques for Sugars') and L-dopa concentration. For the latter analysis an HPLC method is developed analogous to the method of British Sugar (10): a Chromspher 5 C18 column at ambient temperature, 0.04 M potassium phosphate solution at pH=3 with 1% methanol as mobile phase at 0.6ml/min., electrochemical detection using a glassy carbon electrode, injection volume 20µl and no sample preparation (only diluting 25 times).

Laboratory evaporation

Heating experiments of thin juice on a laboratory scale are performed in a stainless steel pressure vessel of 10 litre content in which about 4 litre of juice is heated. An external steam-boiler supplies the necessary energy to the juice via a heating tube inside the vessel; the steam-pressure and thus the temperature can be controlled. During the heating experiment both the steam-condensate and the juice vapour are removed

automatically. Before starting the evaporation, the pH of the thin juice is adjusted to pH 8.7, if necessary, using concentrated sulfuric acid.

The juice in the vessel is subjected to the first three effects in evaporation, e.g. the temperature stages 135°C–125°C–115°C are completed successively. The heating time at each stage is about 8-9 minutes, which is comparable with the average factory practice at CSM of Roberts circulation evaporators. In between the successive heating steps, samples are taken for further analysis. The samples are analyzed for pH, brix, purity, colour at 420nm, invert sugar according to Luff Schoorl, respectively glucose and fructose with the before-mentioned HPAEC method.

RESULTS AND DISCUSSION

Factory measurements

Diffusion

With respect to sugar degradation of microbiological, enzymatic and chemical origin in the diffusion systems of CSM one is referred to an earlier model study of De Bruijn, *et al.* (3). It was concluded that in the extraction systems of CSM chemical and enzymatic sucrose hydrolysis are of equal level. The total sugar degradation due to hydrolysis in the diffusion roughly lies between 0.01 and 0.03% on beet. Normally microbiological sugar breakdown, as indicated by L-lactate in raw juice, is of minor importance in CSM factories because of a tight disinfection régime.

Sucrose hydrolysis is undesirable because of two reasons: it causes loss of sugar and it is one of the major sources of colour in process, e.g. by alkaline degradation and Maillard reaction. Apart from invert sugar, the phenolic compounds are considered to have a large influence on colour formation (9, 10). Particularly the design of the extraction system, e.g. tower or RT diffuser, determines the dissolved oxygen in the juices, and so the completeness of the polymerization reaction of phenolic compounds. The polymerization starts with the oxidation of tyrosine (extracted from the sugar beets) to L-dopa catalyzed by polyphenol oxidase. Then, further oxidation reactions take place leading to dark polymers called melanins, which are considered to be removed by filtration of the first carbonatation precipitate.

The tyrosine content of raw juices from CSM tower diffusers ranges from 100 to 150mg/l, whereas the L-dopa and dopamine concentration is much lower, namely <10mg/l. These data are more or less comparable to those determined by British Sugar in their tower diffusers (10), although the tyrosine content of CSM raw juices is slightly higher and the L-dopa content is somewhat lower.

After juice purification the tyrosine content still is the same as in raw juice, but L-dopa and dopamine have completely disappeared.

Juice purification

During the campaign we have measured the juice colour several times at the different stages in juice purification; i.e. first carbonatation, juice after the clarifier at the inlet of second carbonatation, second carbonatation, respectively thin juice before and after sulfitation. The colour increase of the juice in the purification process throughout the campaign is more or less the same; the colour formation in both CSM factories is also comparable to each other. However, it turned out that the basic colour level may be different every day or even every hour. For example, the colour of the first carbonatation juice varied between values as low as 900 I.U. and as high as 1700 I.U. without any visible reason.

Figure 1 gives the average results of the colour measurement in the juice purification of the CSM factories. An acceptable colour increase of 150-200 I.U. is observed from first carbonatation to thin juice in both factories. After addition of sulfur dioxide, for purpose of pH adjustment and control of colour formation in evaporation, the colour decreases by about 150 I.U.

Recirculation of juices to juice purification, e.g. the filtrate of rotary vacuum filters, the regeneration fluid and washing juice of decalcification resins, collected juices from leakages, may contribute to the final thin juice colour as well. The estimated contribution of recirculated juices to thin juice colour is 100-200 I.U., particularly due to the retention of these juices in buffer tanks at high temperature and relative high pH values.

Evaporation

The difference in thin juice and thick juice colour gives the colour increase in evaporation, which is the consequence of high juice temperatures, particularly in the first evaporator effects. The average data for the last decade are presented in Figure 2.

There are two major differences between the factories:

1. The colour increase in factory 1 is much higher than in factory 2; on the average 30% versus 8% colour increase from thin to thick juice.
2. The thin juice colour of factory 2 is generally lower than in factory 1; on the average 1000 versus 1300 I.U.

The explanation for these differences have to be found mainly in the different ways the diffusion, juice purification and evaporation are operated in both factories, rather than beet quality. The first two process steps will largely determine the thin juice colour, whereas the course of temperature, retention time and pH in the evaporators will influence the colour formation upon evaporation of the thin juice. The origin of the thin juice colour is dealt with further on in this paper at the discussion of the laboratory juice purification experiments.

Figure 3, 4 and 5 give more detailed information with respect to the chemical changes in the evaporator systems. In Figure 3 the gradual colour increase along the subsequent stages in evaporation is demonstrated. Again the difference between the factories is striking.

Due to the heating and evaporation of water, ammonia and carbon dioxide are eliminated from the juice with the vapour phase, which may influence the course of the juice pH markedly (2, 11, 13). Because more than 90% of both volatile components are eliminated from the juice in the first evaporator effects (13), between the second and third effect is considered to be the most efficient place for pH control by the addition of caustic soda or magnesium oxide. This is common practice in CSM factories, which results in only slight pH changes of the juice during evaporation as demonstrated by Figure 4. In order to prevent the often observed fall in pH during evaporation, also a supply of alkali in advance of the evaporation process can be considered, for instance by addition of caustic soda or soda ash to second carbonatation.

Figure 5 illustrates the invert sugar formation by hydrolysis of sucrose during evaporation. Although the course of pH is similar for both factories, with a slightly lower thick juice pH in factory 2 (see Figure 4), the invert sugar increase in factory 1 is substantially higher. Moreover, also in the second step of evaporation there is a considerable amount of invert sugar formed, whereas in factory 2 there is no further hydrolysis of sucrose after the first effect. The main source of the observed differences between the two factories with respect to both colour and invert sugar formation, is the difference in the juice temperature at the time of the measurements, particularly in the first evaporator effect; namely 139°C in factory 1 respectively 134°C in factory 2. This conclusion is confirmed below by the results of the laboratory juice heating experiments. By the way, the above-mentioned high juice temperature in the first evaporator effect has to be considered as a temporary, extreme situation, but it is not common practice in our factories.

The development of the invert sugar content of the juices showed in Figure 5 is determined using the (overall) Luff Schoorl method, which does not necessarily give the true value for the total amount of glucose and fructose present. For purposes of comparison we have analyzed the invert sugar in juices from the evaporation both by the Luff Schoorl method and by HPAEC; the results of one such measurement is summarized in Table 1. These results agree with repeated measurements on other days at both locations, but they will not be presented here.

Considering the differences between the Luff Schoorl and the HPAEC measurements as well as the glucose/fructose ratio the following remarks can be made:

1. The glucose+fructose content remains constant after the second effect, whereas the invert LS content still increases slightly thereafter. Apparently, there is an increase of reducing material, possibly in coloured components, without the formation of invert sugar.
2. The Δ glucose/ Δ fructose ratio in evaporation is ~ 1.6 , which is already valid after the first effect. In other words, to 1 mole of glucose formed only 0.6 mole of fructose is found.

Based on the foregoing remarks it seems reasonable to suppose that after the second effect both the hydrolysis of sucrose and the degradation of invert sugar are negligible. In the first two evaporator effects,

fructose is converted/degraded to some extent; at least 0.4 mole fructose per mole hydrolyzed sucrose has disappeared.

By using HPLC analyses, we have checked for the presence of products which may be expected to be formed from invert sugar under the evaporation conditions. However, we failed to detect either any alkaline or acid degradation products, e.g. lactic acid and hydroxymethyl-furfuraldehyde, or the formation of kestose as a result of the fructosyl transfer to sucrose upon hydrolysis. Consequently, the degradation of fructose via Maillard reaction seems the most likely. Some evidence can be found in the literature that in diluted aqueous solutions, fructose reacts more rapidly with amino acids than glucose (5, 8, 14).

It is supposed that if in evaporation the increase of the glucose content of the juices is multiplied with the factor 2, a reasonable indication will be obtained of the total sugar loss. It should be noted that at the Sugar Processing Research Institute an investigation is in progress on the identification of a marker compound for the most accurate determination of the sugar loss (6).

Laboratory experiments

Juice purification of model solutions on laboratory scale

Juice purification of artificial raw juices till first carbonatation have been carried out in order to obtain more insight into the origin of thin juice colour. As this study is currently underway, only some preliminary results can be presented at this stage.

In order to simulate the oxidation of tyrosine in the factory diffusion into melanins, we have added a pre-oxidized solution of L-dopa to the artificial raw juice. The advantage of L-dopa is that no tyrosinase enzyme is required to catalyze the first oxidation step. Just by aeration of a L-dopa solution, an appropriate oxidation of phenolic compounds can be obtained. Initially we have standardized the oxidation of L-dopa by the following procedure: 250mg L-dopa in tap water is oxidized at 75°C for 20 minutes and the coloured reaction mixture is added immediately to 3 litre of artificial raw juice. The amount of 83mg (partly) oxidized L-dopa in 1 litre of juice is just a rough estimate of the factory practice, but sounds plausible when it is related to the remaining tyrosine and L-dopa contents after the diffusers of 100-150mg/l and <10mg/l respectively in raw juice, as was mentioned above.

The results for the colour of the different first carbonation juices obtained in the laboratory purification experiments are summarized in Table 2.

From the experiments carried out thus far the following preliminary conclusions can be drawn:

1. An invert sugar content of 1.5g/l, which is on the high side of what is normally present in raw juice, results in a colour formation of 90 I.U. and agrees with earlier findings (9). By doubling the invert sugar concentration the colour increases by an additional ~210 I.U.

2. Addition of glutamine or γ -amino butyric acid appears to have only a small effect on the juice colour of 30-40 I.U., so the role of the Maillard reaction in juice purification seems to be limited, even at rather high concentration (1.0g/l) of these amino-components. Our results do not confirm those of Madsen et al. (9), who claimed an increase of 360 I.U. upon addition of a similar amount of glutamic acid.
3. The addition of L-dopa has a considerable effect on the juice colour, but especially the way the pre-oxidation of L-dopa is carried out appears to be decisive in the juice colour finally obtained. Addition of the standard pre-oxidized L-dopa solution to the artificial juice gives rise to a colour increase of 150 I.U. after purification, whereas pre-oxidation in the artificial juice during heating is responsible for a contribution of 340 I.U. to the juice colour.
4. The addition of iron does not further enhance the colour formation by polyphenolics as was expected from literature data (9).
5. Addition of pulp and green beet material caused the colour to increase by 110 I.U.; the individual influence of both components has to be determined in separate experiments.
6. The difference of juice colour between main liming and first carbonatation reflects the adsorption of colouring matter to the calcium carbonate precipitate.
7. Laboratory juice purification of a factory raw juice yields a juice colour of 1300 I.U., which is equal to the first carbonatation juice colour of the factory and thereby underlines the usefulness of the laboratory purification method.
8. Laboratory juice purification of artificial juices with a composition (to our present knowledge) as close as possible to that of raw juice, results in a colour of the first carbonatation juice which is still far below the colour usually produced in our factories (colour range 900-1700 I.U.).

Up to now we have not been able to close the gap between laboratory and factory juice purification with respect to colour formation. Further research will be needed in order to elucidate the combined role of the different components involved in colour formation. The oxidation of polyphenolic compounds appears to be of the utmost importance in this respect.

Heating of thin juice on laboratory scale

A thin juice sample, which was taken after sulfitation, has been treated several times in three subsequent heating steps with the temperature range as a variable. The course of the colour and glucose + fructose in these experiments is depicted in Figures 6 and 7.

It should be noted that in these trials it was not possible to control the pH, which caused a moderate pH decrease when the first heating step was carried out at 140°C or 135°C. The final pH after three heating steps dropped to 7.9 and 8.4, respectively, which certainly will have resulted in some extra sugar hydrolysis.

After the three heating steps a dry substance content of generally ~21% is obtained, already achieved in the second evaporator effect of the factories.

Nevertheless, the experimental data clearly demonstrate the correlation between a high evaporation temperature, resulting colour formation, and sugar hydrolysis. A juice temperature $\leq 135^{\circ}\text{C}$ in the first evaporator effect is recommended in order to prevent excessive invert sugar and colour increase of the juice.

Figure 8 shows the glucose + fructose increase after one heating step at different temperatures. It should be noted that also in the laboratory heating experiments the $\Delta\text{glucose}/\Delta\text{fructose}$ ratio appears to be ~1.6. The dotted line represents 2 times the glucose content and thus gives the best possible approach of the total sugar loss in the first step. For example, the increase of glucose + fructose, i.e. $\Delta(\text{G}+\text{F})$, of slightly above 0.2g/100g d.s. at 139°C in the model experiments is reasonably good comparable to the factory practice, where we have found an increase of 0.16g/100g d.s. after the first evaporator effect (see Table 1).

Finally, Figure 9 demonstrates the influence of the temperature in the first heating step on colour formation. Additionally, the dotted line in Figure 9 represents the colour increase of thin juice upon heating in case there is no sulfur dioxide present. It can be seen that in thin juice with sulfur dioxide (SO_2 -level 120-150mg/kg) the colour increase develops exponentially and becomes rather important above 135°C . In the absence of sulfur dioxide the relation between colour increase and temperature shifts down with about 10°C .

CONCLUSIONS

Sugar degradation

The ins and outs of sugar degradation in diffusion, juice purification and evaporation are quite well understood nowadays. Modern liquid chromatographic analysis techniques have been very helpful in this respect. There still remains some doubt as to the true sugar loss in evaporation since both the hydrolysis of sucrose and the degradation of the invert sugar formed proceed simultaneously. Analysis of the glucose increase in the juice during evaporation and multiplication of the result by a factor of 2 is the best possible estimate of the sugar loss in evaporation up to now.

The increased knowledge on sugar degradation has been successfully applied by CSM to control the process more properly, e.g. disinfection and pH-control in diffusion, pH-control and temperature régime in evaporation.

Colour formation

Factory measurements concerning juice colour have revealed that thin juice colour and colour increase in evaporation may be different among factories. In addition, thin juice colour may vary significantly within one factory from day to day or even from one hour to the next. By comparison of laboratory heating experiments and factory practice it is concluded that especially the temperature of the first evaporator effect determines the extent of colour formation in evaporation. With respect to both colour increase and sugar

hydrolysis in evaporation a juice temperature below 135°C in the first evaporator effect is recommended, which can be achieved by the installation of sufficient surface for heat-exchange.

A study on the origin of thin juice colour and the causes of its variability is currently in progress. From some preliminary experiments the main components in raw juice responsible for the colour after juice purification could be ascertained, namely invert sugar, (partly) oxidized polyphenolic compounds, pulp and/or green beet material. Further research will be required in order to close the gap between juice colour obtained by laboratory purification of artificial raw juice and juice colour in sugar manufacture.

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Table 1. Invert sugar Luff Schoorl (inv.LS), respectively glucose (G) and fructose (F) using HPAEC in evaporator juices: concentrations in g/100g d.s.; Δ is the increase in comparison with thin juice.

juice	glucose	fructose	$\Delta G/\Delta F$	G+F	invert LS	$\Delta \text{invLS}/\Delta (G+F)$
thin juice	4	2		6	6	
1st effect	14	8	17	22	29	14
2nd effect	17	11	14	28	40	15
3rd effect	17	10	16	27	41	17
4th effect	17	10	16	27	43	18
thick juice	17	10	16	27	43	18

Table 2. Results of colour measurement after laboratory juice purification of artificial juices.

Experiment	Composition of artificial raw juice; additions to the blank ¹ juice	Colour I.U. main liming	Colour I.U. 1st carbonatation
1	-	-	20
2	Inv	-	110
3	2*Inv	-	330
4	Inv + glutam	310	140
5	Inv + gaba	-	150
6	Inv + dopa (stnd ox)	540	260
7	Inv + dopa (stnd ox) + Fe	740	240
8	Inv + 3*dopa (stnd ox)	1150	16502
9	Inv + 6*dopa (stnd ox)	3210	23102
10	Inv + dopa (stnd ox) + P + GM	800	370
11	Inv + dopa (stnd ox) + P + GM + glutam	610	410
12	2*Inv + dopa (stnd ox) P + GM	1050	570
13	Inv + dopa (ox in juice)	950	450

¹ Components added to the blank juice (see Methods and Materials for their concentrations), in which sucrose and a mixture of organic acids and salts are present:

Inv glucose and fructose

glutam glutamine

gaba γ -amino butyric acid

P pressed pulp

GM green beet material

dopa L-dihydroxy phenylalanine: 'stnd ox' means pre-oxidation of dopa as mentioned above; 'ox in juice' means that dopa is pre-oxidized during the heating of the artificial juice

² After the first carbonatation the sample continues to develop colour due to further oxidation of unremoved and still reactive phenolic compounds.

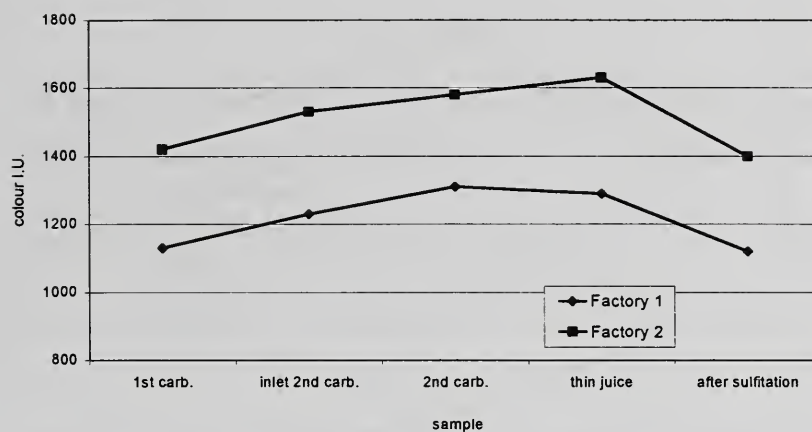


Figure 1. Colour development in juice purification - at CSM factories.

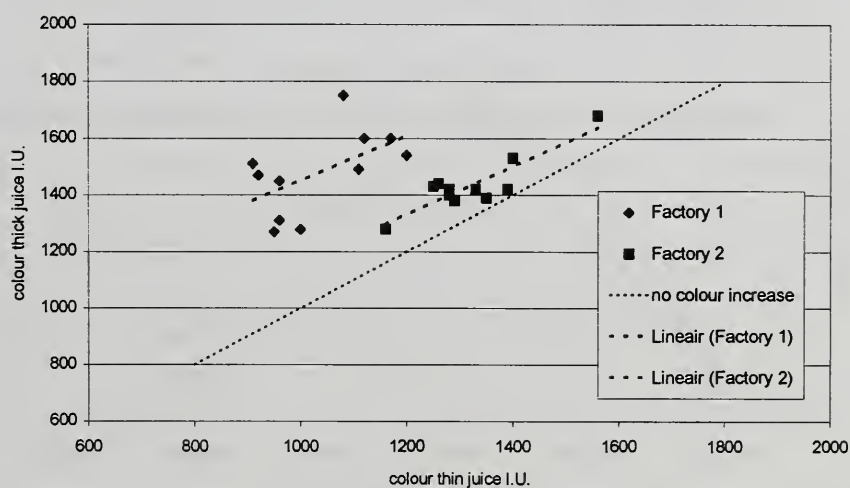


Figure 2. Colour increase in evaporation - averages of different campaigns.

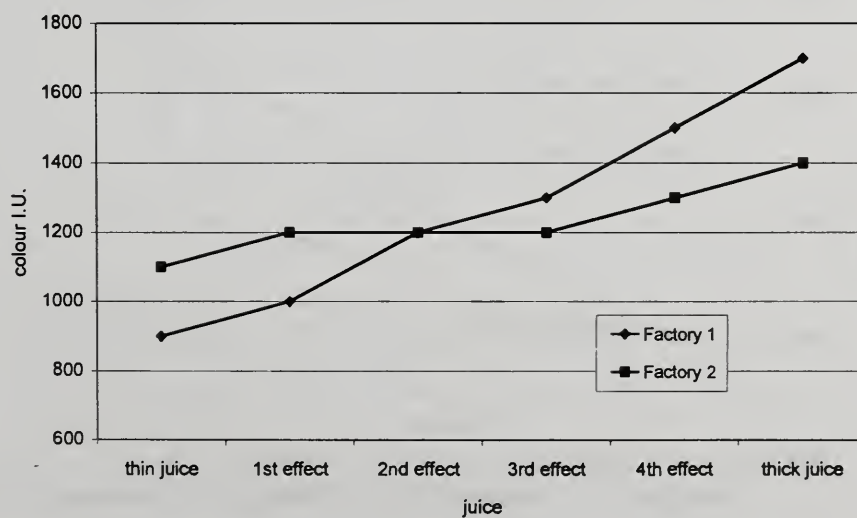


Figure 3. Colour increase in evaporation.

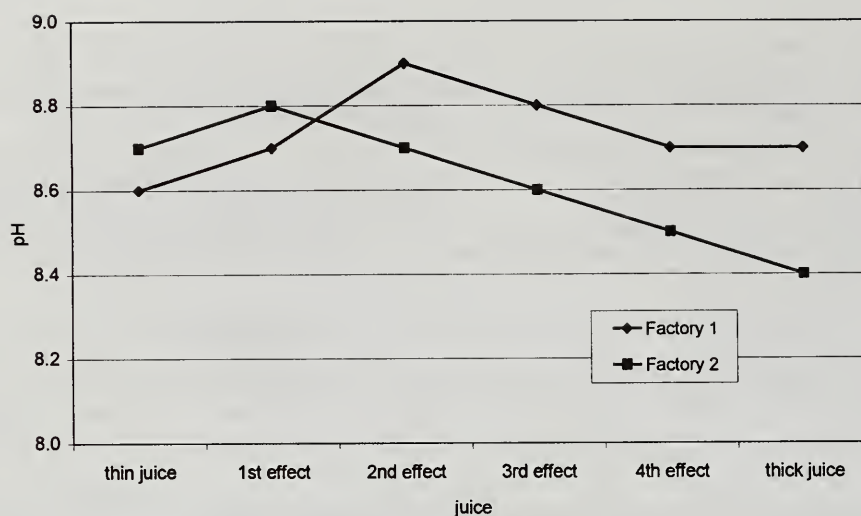


Figure 4. Course of pH in evaporation.

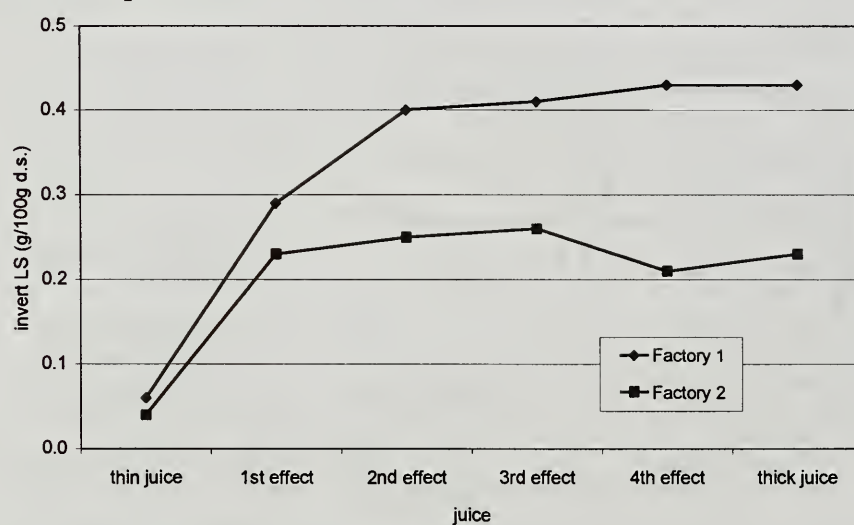


Figure 5. Invert sugar formation in evaporation.

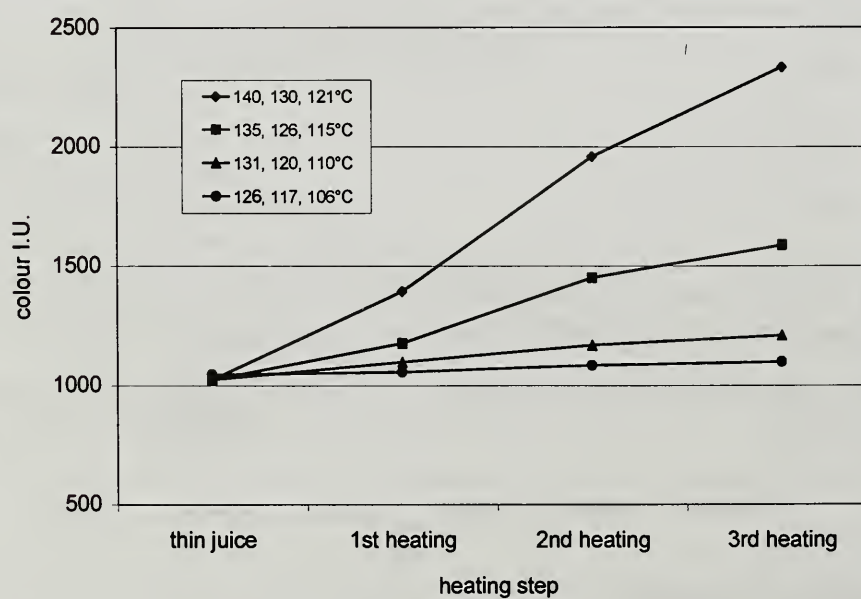


Figure 6. Colour increase in successive laboratory evaporations.

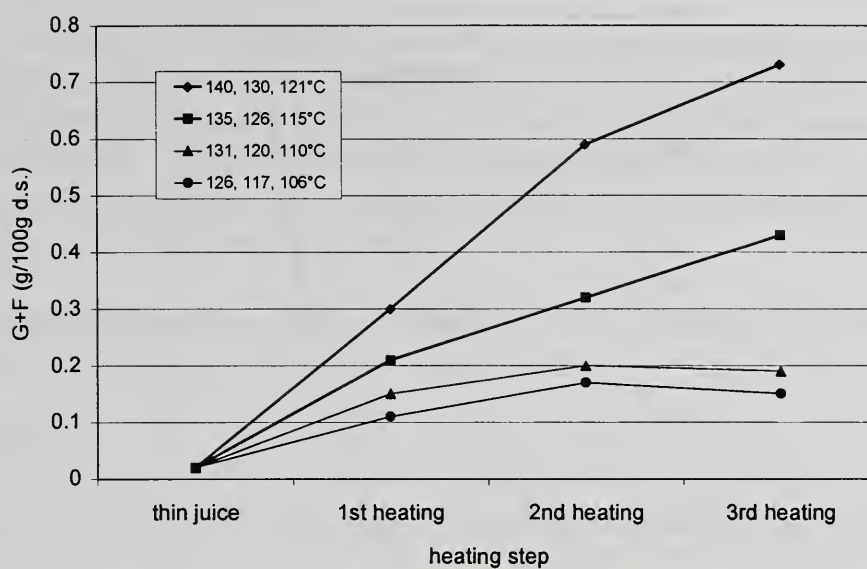


Figure 7. Increase of glucose + fructose in successive laboratory evaporations.

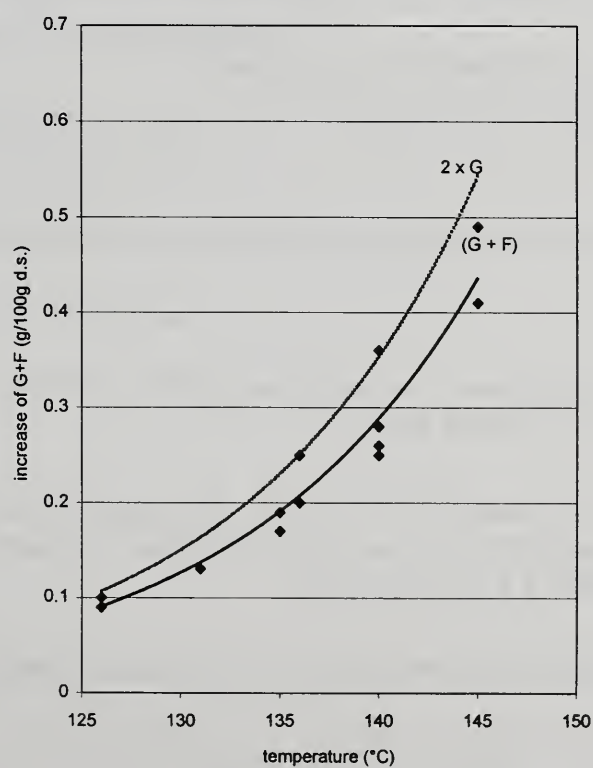


Figure 8. Increase of glucose + fructose in the first laboratory heating step.

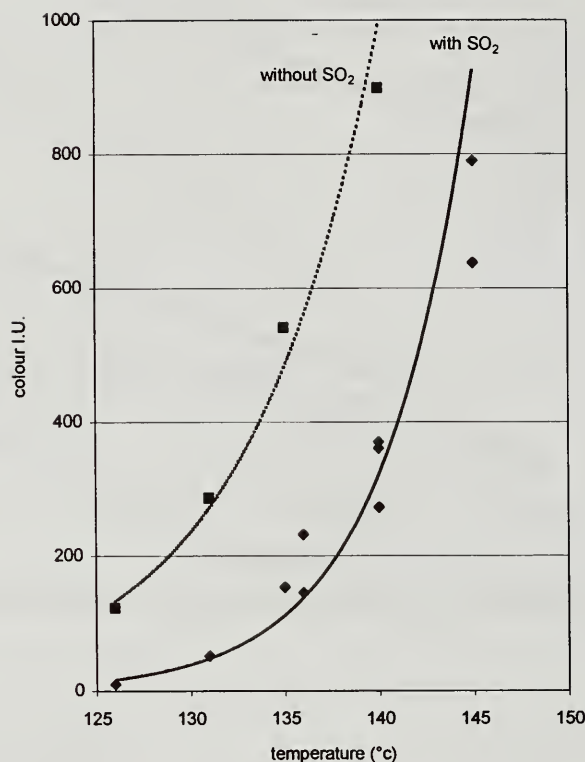


Figure 9. Colour increase in the first laboratory heating step.

DISCUSSION

Question: On your evaporator studies have you an optimum pH for the least amount of color formation and sucrose degradation from thin juice to thick juice?

de Bruijn: There is a very narrow range that is optimal. The higher pH is better for preventing sucrose degradation in terms of hydrolysis, but to prevent color formation, it is better to have a lower pH. I think this is between 8.5 and 9. Probably 8.7 is the best pH.

Question: How about when you go to the pans, like the white pan, the intermediate pan and so forth? Any studies on pH there?

de Bruijn: The pH in the white pans is between 8.0 and 8.5 and may drop to 7.5-8.0 in the after product massecuite.

Question: Would you have degradation at lower pH's, say at 7.5?

de Bruijn: Yes, but the extent will depend on temperature and retention time as well.

Comment: I notice that the invert formation across the evaporators was very similar to a study I did in another European factory, and I just wanted to make a comment. The reason is that at the beginning the thin juice is at a higher temperature and has a low Brix. So there will be a higher dissociation of sucrose in the water, which provides protons. That is the reason for the acid hydrolysis reactions producing glucose and fructose. As you go across the evaporator, the temperature goes down, the Brix goes up, and there are less protons available. That is why you see the changes.

Question: In your model studies, what form of iron did you use? Because at elevated pH, you will have some problems with iron salts being insoluble.

de Bruijn: It was Iron III.

Question: In your two factories, are the pH controls the same, and are the evaporator systems the same?

de Bruijn: The evaporators are the same (Roberts) type. pH control between the 2nd and 3rd body by addition of caustic soda are similar in both factories, but sometimes it is necessary to anticipate too large pH changes in evaporation by addition of some caustic soda or soda ash to the juice purification.

Comment: I am glad you mentioned that because your work on alkaline hydrolysis is the foundation work for everybody these days. It is the main reference.

I am sure that you are aware that the G/F ratio, which you mentioned, is almost entirely controlled by pH. If anybody is aware of that, it should be you.

Question: What is the approximate SO_2 concentration in thin juice where you saw the lowest color increase?

de Bruijn: At SO_2 -level of 120-150 mg/kg.

OVERVIEW OF NEAR INFRA-RED SPECTROSCOPY(NIRS) RESEARCH IN THE SOUTH AFRICAN SUGAR INDUSTRY

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ABSTRACT

Sugar industries world-wide are continuing to show increasing interest in the potential applications of near infra-red (NIR) analysis as a research and management tool in the fields of soil fertility, cane nutrition, cane quality testing, and in the possibility of screening for resistance to certain pests and diseases. In South Africa during the past decade both filter and scanning NIR reflectance spectrophotometers have been used to improve nitrogen use efficiency of sugarcane by matching the crop's N requirement to soil N mineralising potential and plant N status, both properties being determined by NIR. Calibrations were developed and validated for N in leaf as well as total N content, organic matter content, N mineralisation potential, and texture of soil samples. Well over 80,000 leaf and 200,000 soil samples submitted by cane growers have been routinely tested by the Fertiliser Advisory Service (FAS) using NIR in conjunction with other instrumental techniques. Major benefits have been substantial savings in N fertiliser use as well as a reduction in the risk of environmental pollution. Recent developments have centered on comparing the suitability of both filter and scanning instruments for the rapid determination of various constituents in cane juice, shredded cane, bagasse, raw sugar, and molasses. The possibility of using NIR on shredded cane has been proposed as an alternative to direct analysis of individual cane consignments (DAC) following collaborative investigations by staff from the Sugar Milling Research Institute (SMRI) and Sugar Experiment Station (SASEX). Global calibrations developed for mixed cane juice and molasses constituents for daily process control management of raw sugar factories are also currently under investigation by SMRI staff. Possible new applications for NIR that are discussed include partitioning the N pool in the cane plant, estimating photosynthesis, predicting yield potential and screening for pest and disease resistance.

INTRODUCTION

The use of near infra-red spectroscopy (NIRS) for the analysis of products in the food, chemical engineering, biochemical, environmental, pharmaceutical and medical fields has increased considerably during the past decade. The success of NIRS can largely be attributed to its ability to conduct rapid quantitative and qualitative analyses of multi-components in single samples using minimal sample preparation. Despite advances in applying NIR at the research and process levels for cereals, oilseeds, forage assessment and sugar beet analysis, relatively little progress has been made in adopting this technology for routine use in the cane industry. Research has mainly been confined to the USA, Australia and South Africa, and includes using both filter and scanning instruments for foliar diagnosis [1], N fertiliser management [2], cane juice analysis [3,4], shredded cane analysis [5-9] assessing soil properties [10], analysis of sugar related products [11,12], and predicting varietal resistance to the stalk borer *Eldana saccharina* Walker (Lepidoptera : Pyralidae) [13].

This paper is a summary of some past and current research in South Africa, as well as indicating potential new applications. Comparison of NIR spectra for a range of products tested are shown in Figure 1, while Table 1 is a summary of the constituents for which successful calibrations have been developed.

SUGAR CANE NUTRITION

The South African sugar industry greatly depends on soil and leaf analysis conducted by its Fertiliser Advisory Service (FAS), for identifying and correcting nutrient disorders in sugarcane. The determination of the nitrogen requirement of sugarcane is one of the more important activities undertaken by the Sugar Experiment Station. Of the sixteen elements considered to be essential for sugarcane, nitrogen has the greatest effect on cane growth and juice quality. The importance of N to the economy of sugarcane may be judged from the fact that over 30,000 tons of N, valued at \$25 million are used in the South African sugar industry each year. Traditional methods of nitrogen analysis used in formulating N recommendations are not only time consuming but also very labour intensive. Details of calibrations developed for selected products and constituents are summarised in Table 1.

Leaf tissue analysis

In 1983, a much improved system of leaf N analysis was introduced when a Technicon 300 Bran filter instrument was first calibrated and validated for leaf N analysis [1]. Fifty leaf samples with N contents ranging from 0.80-3.0% were used to calibrate the instrument. A further 125 samples analysed by the standard Kjeldahl steam distillation method were used to validate the N calibration (see Table 2). The accuracy and precision of the NIR method was further evaluated by repeatedly analysing 13 reference samples over a period of five days. The small differences between the mean results (0.07%), and the small variation in the N values obtained (CV range 0.8-4.5%), suggested that the NIR method was sufficiently reliable for determining N in leaf samples. In practice, a level of accuracy of $\pm 0.1\%$, and reproducibility below 5%, are considered to be acceptable in assessing the N status of sugar cane by means of leaf analysis. The method was also shown to be about ten times faster than the steam distillation Kjeldahl procedure for N. Typically a batch of 75 leaf samples could be analysed by a single operator in about 60 minutes. NIR also resulted in a labour saving of at least eight man hours per 200 samples.

Subsequent investigations using a Foss NIR Systems 5000 scanning spectrometer and ISI software, has led to more reliable calibration equations for leaf N and also acceptable equations for determining other nutrients such as P, K, Ca, Mg and S.

Fertility trend analysis

Some of the more important applications of NIR in leaf analysis concern its use in controlling whole crop cycle recommendations and in nutrient survey programmes. Since 1980 more than 80,000 leaf samples have been analysed for N content by NIR. The data set is regularly updated and used to determine comparative changes in nutrient availability in the sugar industry [12]. The average N level has steadily declined with

the incidence of N deficiency increasing from 10 to 26% between 1980 and 1997. This increase in N deficiency is considered mainly due to rationalisation of fertiliser use during the 1993-1995 drought as well as the continuing threat of *Eldana saccharina* Walker stalk borer, which is known to cause more damage at higher levels of N fertilisation. The decline in leaf N content may also be attributed to the increased use of varieties N12 and N14 which are both known to have a relative lower N uptake pattern than NCo376.

Cane areas with the lowest average leaf N content and the highest proportion of samples deficient in N included Kwa Zulu small scale growers (57%), Zululand South (40%), North Coast small scale growers (33%), North Coast (27%), Zululand North (27%) and South Coast (25%) These areas are also associated with the largest areas of low N mineralising soils (Category 1) that have a low potential for supplying the crop with N released from soil organic matter. Also the organic matter content, which is the main native source of mineral nitrogen in the soil is slowly degrading under a system of cane mono-culture. Degradation of soil organic matter is a major problem on the muck soils in Florida.

N use efficiency studies

NIRS has provided a rapid means of detecting, through leaf analysis, the relative efficacy of timing, placement and the use of different N carriers in various trials [13]. Recently leaf NIRS analyses proved useful in assessing the N requirements of different cane varieties. For many years, fertiliser recommendations in the South African sugar industry were based on the variety NCo376. Analyses of the standard top visible dewlap (TVD) leaf, covering thousands of samples from variety trials, have shown significant varietal differences in N content. Current trials indicate that the optimum N requirement of important varieties such as N12 and N14 can differ from that of NCo376 by up to 75 kg N/ha. Limited field evidence also suggests that a variety such as N12, which uses N less efficiently than NCo376, may respond more effectively to split N applications. Threshold levels for interpreting leaf analyses may require adjustment for varieties that use N less efficiently.

SOIL FERTILITY STUDIES

Nitrogen mineralisation potential

Soil organic matter (SOM) is widely regarded by soil and environmental scientists as one of the most important properties of soil. Not only does it promote physical properties such as soil structure, intake rate and water holding capacity but it also regulates the carbon, nitrogen, phosphorus and sulphur balance in soils. Research in South Africa has shown that SOM through N mineralisation can supply between 20 to 90% of the N that the crop takes up.

In 1965, Bower and Hanks studied the reflection of radiant energy from soils and found that organic matter, moisture content and particle size affected the amount of radiant energy reflected from soils. Following the successful application of NIR to N analysis of cane leaf samples, attention was given to using NIR for soil testing. Previous work in the South African sugar industry had shown that the N requirement of sugarcane could be estimated more reliably from soil properties such as N mineralisation potential, texture, colour and

organic matter content. For advisory purposes a system was developed for placing soils into low, moderate, high and very high mineralising categories [2]. Two hundred air-dried ground soil samples (0.25 mm sieve), of known mineralising potential, organic matter, total N, and clay content were used to calibrate a Technicon InfraAlyzer 450 instrument. Comparative statistical information obtained for these different constituents (Table 2) suggests that most of these properties could be satisfactorily estimated by NIRS. Reliability decreased in the order: clay, organic matter, total nitrogen and N mineralisation rating [10]. Coded soil samples from 21 N trials showed that predicted N mineralization ratings were correct in 17 of the trials.

More recently a Foss NIRS Systems 6500 scanning instrument was used to capture diffuse reflectance spectra at 2nm intervals of composite air-dried ground topsoil samples (0.25mm sieve), representing 20 soil forms of varying organic matter, total N, clay, silt, sand and fertility status. Preliminary results suggested that NIRS provides acceptable calibrations for determining soil pH, acid saturation, base status, cation exchange capacity and P fixation using a P desorption index (PDI).

SUGAR PRODUCTS

Analysis of pol and Brix in sugarcane is an important analytical service rendered by laboratories in the sugar industry. The standard procedure based on filtration and clarification of expressed cane juice is tedious and labour intensive. In 1987, the suitability of NIRS for rapidly estimating cane juice quality components was assessed [3]. Mixed cane juice samples of known pol, Brix, sucrose, fructose and glucose contents were used to calibrate a Technicon InfraAlyzer 450. Regression analyses indicated that Brix, pol and sucrose values by NIRS were closely correlated with those obtained by conventional methods of analysis.

In 1992, an NIRSystems 6500 spectrometer was used to study analytes in bagasse, shredded cane, direct analysis of cane (DAC) extracts, mixed juice, molasses and raw sugar [11]. Analytes included pol, Brix, dry solids, moisture, sucrose, glucose, fructose, invert, ethanol, colour, ash and starch. NIR produced a good estimate for many of the analytes tested (Table 2).

More recently an intensive collaborative investigation between the SMRI and SASEX, used >500 shredded cane samples to calibrate and validate a NIR System 5000 spectrophotometer for pol, Brix and moisture readings [9]. Calibration equations developed at one mill in 1994 were evaluated using data from four other mills over a period of two different seasons (1992,1995). Calibration R values based on partial least squares regression analysis were better than 0.97 for Brix and pol, but lower for moisture (0.945) (see Table 3). Validation of the calibration showed that the NIR predictions for pol, Brix and dry matter were generally satisfactory with R values of 0.91-0.96 (see Table 4).

These results were particularly pleasing as calibrations and predictions were carried out on different NIR instruments and the original laboratory data were produced by four different laboratories. Furthermore, application of the calibration equations developed from the commercial cane samples to clean shredded samples from clonal evaluation trials also showed good correlation between predicted and laboratory measurements, but there was a significant bias of almost one unit between the two sets of measurements due mainly to spectral differences between clean and dirty industrial cane. A small part of the bias (0.20%) was also due to differences in the extraction efficiency of the methods used between the SASEX and CTS

laboratories. The mere fact that NIR was able to detect the bias between the two laboratory methods is a strong indication that NIR can be used for shredded cane analysis. In general, it was concluded that the technique was sufficiently reliable for rapid analysis of sugarcane in plant breeding and agronomy variety trials. SMRI staff are continuing to evaluate NIRS as an alternative to direct analysis of growers' cane consignments in the millyard using shredded cane samples from both North and South Coast mills.

In the most recent investigation carried out by SMRI staff [12], over 550 mixed juice and over 900 C-molasses samples covering four seasons were scanned from 1100-2400nm. The mixed juice samples were randomly split into two sets. Partial least squares regression, together with principal components analysis, were employed to develop calibrations for Brix, pol, sucrose, glucose, fructose, ash and purity in one of the two sets. The resulting calibration equations were validated against the remaining set of samples. NIR calibrations and predictions for sucrose, pol and Brix in mill juice were most impressive ($SEP < 0.1$, $RSQ=1$, $bias=0$, $slope=1$). The NIR-SEP's obtained for sucrose, pol and Brix (0.09, 0.08 and 0.10) were remarkably similar to the precision obtained for the reference method used by CTS laboratories. NIR estimates for Brix and dry solids in molasses were also excellent, with SEP similar to the precision of the reference method. The global calibrations developed by SMRI will be used to initially calibrate a Foss NIRS Systems 5000 spectrometer at one South African mill for the 1997-98 season when the accuracy of NIR for both mill juice and molasses under factory conditions will be assessed.

FUTURE APPLICATIONS

Estimating photosynthetic rates

Measuring photosynthesis in the field is time consuming, weather dependent and requires considerable skill. Photosynthetic rates were measured on 70 leaf samples from a variety trial using a portable infra-red gas analyser [14]. The samples were scanned (1100-2400 nm region) separately in the fresh and dried state using a Foss NIR Systems 500 instrument. Step-wise regression analysis showed that photosynthetic rate and internal CO_2 concentrations were highly correlated with NIRS absorption values ($R > 0.95$). The wavelengths selected for the calibration equation (2139 nm, 2100 nm and 2190 nm) were consistent with the third overtone stretching vibrations of C=O, O-H and C-H bonds associated with carbohydrate compounds, as well as second overtone N-H bending modes found in proteins. Various investigators have demonstrated a positive correlation between leaf photosynthetic rate, chlorophyll and soluble protein content [15,16]. Recently, leaf photosynthesis in soya beans was positively correlated with leaf greenness, measured nondestructively by means of a hand held portable chlorophyll meter [17].

Examination of the NIRS data showed that 30% of the variation in photosynthetic rate could be accounted for by variation in leaf N. Photosynthetic response to increasing light intensity is strongly dependent on leaf N [19,20]. Inherent differences in yield potential between varieties in many crops may be due to differences in N use, which in turn determine radiation use efficiency [21]. The other characteristic that accounted for a significant variation (37%) in photosynthesis was leaf Si content. It has been shown, under normal light, that silica deposited in silica cells and stomatal guard cells could serve as "windows" allowing more light to pass through the epidermal to the photosynthetic mesophyll tissue [22], thus promoting higher rates of photosynthesis and more tillers per plant. This could partly account for the significant relationship that was

obtained between cane yield and leaf ash content in the Pongola data set ($R=0.68$), as silica comprises about 70% of the ash in sugarcane.

Predicting yield potential

NIR has the potential to detect key constituents such as starches, sugar, cellulose, lignin, proteins, water and amides and also certain constituents linked with S, Mg, Ca and K. Foliar diagnosis relies on the principle that if nutrient concentrations are below the critical level or the nutrients are not in balance then the yield potential of the crop may not be realised. A set of 400 top visible dewlap (TVD) leaf samples collected from 20 regional variety trials, located throughout the cane belt, were used to determine possible relationships between yield parameters, leaf composition and NIRS reflectance measurements. Results from irrigated trials at Pongola showed that NIR absorption spectra of leaf samples from 4-5 month old cane, in the 2238-2500 nm range, were positively correlated with cane yield ($R=0.91$). A comparison of yields predicted by NIR at 4 months with actual yields obtained at harvest for variety CP66 from 10 plots is shown in Figure 2. There was a negative correlation between NIR absorption in leaf samples and pol% ($R^2 = 0.82$) in 12 month old cane. Accuracy of the calibration equations was tested on five independent data sets and, although the correlation coefficients for validation were generally lower (0.60-0.86), the results were sufficiently promising to warrant continuing this field of study. A comparison of pol% values that were predicted by NIR at 4 months crop age with the actual pol values measured at harvest for variety NCo376 is given in Figure 3.

Of additional interest is that much of the variation in pol could be accounted for by the N and ash content of stalk and leaf. Previous studies have also shown that high N levels in the leaf could be associated with lower sucrose values [23,24] while high ash levels in cane juice were negatively correlated with sucrose content [25]. NIRS research in Australia has indicated that the ratio of total N to non-structural carbohydrate in whole shoots of wheat may be a key indicator of yield potential [26]. It is possible that this ratio in irrigated young cane also may have a role as an indicator of crop potential.

Predicting host plant resistance to pests and diseases

There is the prospect that NIRS may prove suitable for screening germplasm from breeding material and wild species of sugarcane for resistance to pests and diseases. NIRS was recently evaluated for predicting flavonoid characteristics associated with *Eldana saccharina* resistance [13]. This stalk borer is endemic in South Africa and causes costly damage to cane each year. Multiple regression predictive models based on NIRS data from 30 clones of known *Eldana* resistance suggested that stalk bud scale and wax components accounted for up to 55% of the variation in resistance. Current work is being done to validate these NIRS bud scale and wax resistance models.

Leaf NIRS scanning is also under investigation as a means of predicting host *Eldana*, mosaic and smut resistance in cane. Preliminary results using 230 leaf samples from trials on 12 commercial varieties suggest that up to 60% of the variation in *Eldana* resistance could be accounted for by absorption of constituents in

the NIR region. Further investigation into likely cause and effect relationships suggests that some of the resistance is linked to leaf silicon ($R=0.60$) and nitrogen(0.39) contents.

Leaf Si is a useful indicator of the silicon status of sugarcane [27]. It is possible that Si contributes significantly to stalk borer resistance in sugarcane. In Florida, high Si uptake in sugarcane following treatment with a silicate slag served as a deterrent to the stem borer *Diatraea saccharalis* [28]. Pot trials are currently being used to investigate the association between host plant Si and N and infestation by Eldana. NIRS leaf calibrations for Si have been established. Another possible new application of NIR is for detecting resistance to diseases such as mosaic and smut. Preliminary results with 15 cane varieties have shown that the standard ratings of mosaic and smut were significantly correlated with leaf spectral absorbance in the NIR region.

Plant N Pools

Quantifying the distribution of forms of plant N in sugarcane is another new research area in which NIRS can be used. Forms of nitrogen include nitrate, amino, soluble protein N and proteins having different structures such as proline-rich proteins. Total N is often used to assess N adequacy, but it includes stored N, which is not very mobile. Possibly, as in other crops, nitrate N may be a more sensitive indicator of N sufficiency than total nitrogen. Knowledge of the transformation of proteins in the cane plant from structural to soluble proteins could assist in predicting the effects of N on cane quality, as well as explaining the tolerance of plants to certain pests and diseases. Proline is an amino acid that has been linked to moisture stress [29] and as such could be a useful indicator when interpreting foliar analyses. High proline indicates that the crop was stressed at sampling, and that caution is then needed when interpreting leaf analyses.

CONCLUSIONS

Because NIRS can be used to analyse materials as diverse as soil, plant tissue, shredded cane, cane juice, bagasse and molasses, it is more versatile than any other analytical technique currently available. It has the added advantage of being fast. It is envisaged that in future, NIRS will play an increasingly larger role at SASSEX, especially in the routine determination of quality in samples from Plant Breeding and Agronomy trials. In Brazil, 15 mills which process a total of more than 50 million tons of cane, are using NIRS analyses of juice for cane payment purposes based on technology emanating from South Africa. In the FAS laboratory, all growers soil samples will in future be screened for organic matter content.

A trend that is emerging in the USA and Europe is towards site specific management, where Global Positioning System (GPS) controlled fertilizer applicators can vary the amount of fertilizer applied to a field according to soil test results. It is envisaged that soil based NIR tests from FAS will play an important role in characterising spatial variability of soil fertility across a field while leaf NIR scanning could be used in yield mapping where water is not a limiting factor. This could ultimately compliment NIR remote sensing from NASA's Airborne Imaging Spectrophotometer to determine yield and quality of sugarcane crops, by using NIR calibrations of the crop canopy. Further research in this area would be needed.

In view of the rapid advances being made with portable handheld NIRS units, cane producers may be able to use this technology for checking crop N status, monitoring crop maturity, determining when to apply chemical ripeners and planning field harvesting programmes. Handheld NIRS units with limited spectral ranges are already being used for various agricultural applications in the USA and Australia. Staff at the Yanco Agricultural Institute in New South Wales are currently evaluating a portable system for monitoring rice quality. NIR monitoring of crops also could have inputs into crop modelling, through monitoring N, photosynthesis and crop maturity status, thereby improving the accuracy of crop forecasting.

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Table 1. Summary of working calibrations developed for a range of constituents in various products.

Cane leaf	Soil	Cane juice	Shredded cane	Raw sugar/molasses
N, P, S, Si	Total N	Pol	Moist	Pol
Photosynthesis	Org. C	Brix	Pol	Brix
Yield	Min. N	Sucrose	Brix	Sucrose
Eldana rating	Clay	Glucose	Fibre	Fructose
Mosaic rating	Silt	Fructose	Tannin	Glucose
	Sand	Alcohol	Lignin	Ash
	CEC	Total N	Waxes	Starch
				Invert

Table 2. Examples of calibrations developed for selected constituents. (n = number of samples, R = regression correlation coefficient, SEC = standard error of calibration, SEP = standard error of prediction, nd = not determined)

Product/ constituent	Range	Wavelength (nm)	Calibration			Validation		
			n	R	SEC	n	R	SEP
CANE LEAF			98			125		
N	0.8-3.0	2186, 1240		0.98	0.11		0.96	0.15
Ash	1.0-9.0	1900, 1860, 1968	61	0.97	0.15	94	0.93	0.23
Silicon	0.5-4.0	1900, 1682, 2448	27	0.89	0.05	27	0.77	0.07
Photosyn.	15-40 umol/m ² /s	2100, 2139, 2190	42	0.90	0.84	30	0.65	2.90
Cane yield	85-210 t cane/ha	2384, 2238, 2448	51	0.91	13.00	191	0.86	14.00
Eldana	20-220	2332, 1754, 2320	51	0.75	17.00	191	0.69	18.00
SOIL			200			74		
N min. pot.	1-4	2236, 2230		0.86	0.30		0.83	16.00

Table 2. (continued)

Product/ constituent	Range	Wavelength (nm)	Calibration			Validation		
			n	R	SEC	n	R	SEP
Total N	0.03-0.60	2050, 1870		0.9	0.01		0.84	19
Organic C	0.3-7.0	2050, 1744		0.92	0.46		0.83	19.00
Clay	5-75	1956, 1920		0.94	3.80		0.81	15.00
CANE JUICE			90			35		
Pol	0.7-13.0	2274		0.96	0.25		0.91	3.20
Brix	1.7-14.0	1366, 2160		0.98	0.15		0.92	3.30
Sucrose	0.7-13.0	2322		0.94	0.29		0.90	3.40
Glucose	0.15-0.80	2342		0.65	0.04		nd	
Fructose	0.15-0.80	2292		0.68	0.50		nd	
SHREDDED CANE			26			136		
Brix	6-21	1434, 2082		0.94	0.24		0.85	3.80
Pol	5-19	1198, 2282		0.93	0.42		0.88	3.90
Dry matter	16-38	2224, 1838		0.88	0.86		0.82	4.70
Fibre	7-14	1376		0.85	0.41		0.80	6.00
CANE STALK			32					
Wax	Qualitative	1940, 1194, 2072		0.65	2.10		nd	nd
Bud scale flavonoids	Qualitative	2180, 1734, 1680		0.75	1.70			

Table 3. Summary statistics for the PLS calibration of Brix, pol and moisture from 116 shredded cane samples collected in 1994 from Mt. Edgecome mill (source ref 9).

Analyte	No. of outliers	No. of factors	MSECV*	R	SEC**
Brix	3	11	0.027	0.979	0.290
Pol	4	14	0.036	0.982	0.300
Moisture	2	9	0.093	0.945	0.570

*MSECV = mean square error of cross validation

**SEC = standard error of calibration

Table 4. Prediction statistics for analytes in the 1992 shredded cane samples (Maidstone mill) using calibrations developed in 1994 (Mt. Edgecombe mill) (source ref 9).

Analyte	Bias	Slope	R	SEP
Brix	0.1	1.100	0.96	0.33
Pol	-0.9	0.999	0.94	0.52
Moisture	-0.4	0.910	0.91	0.90

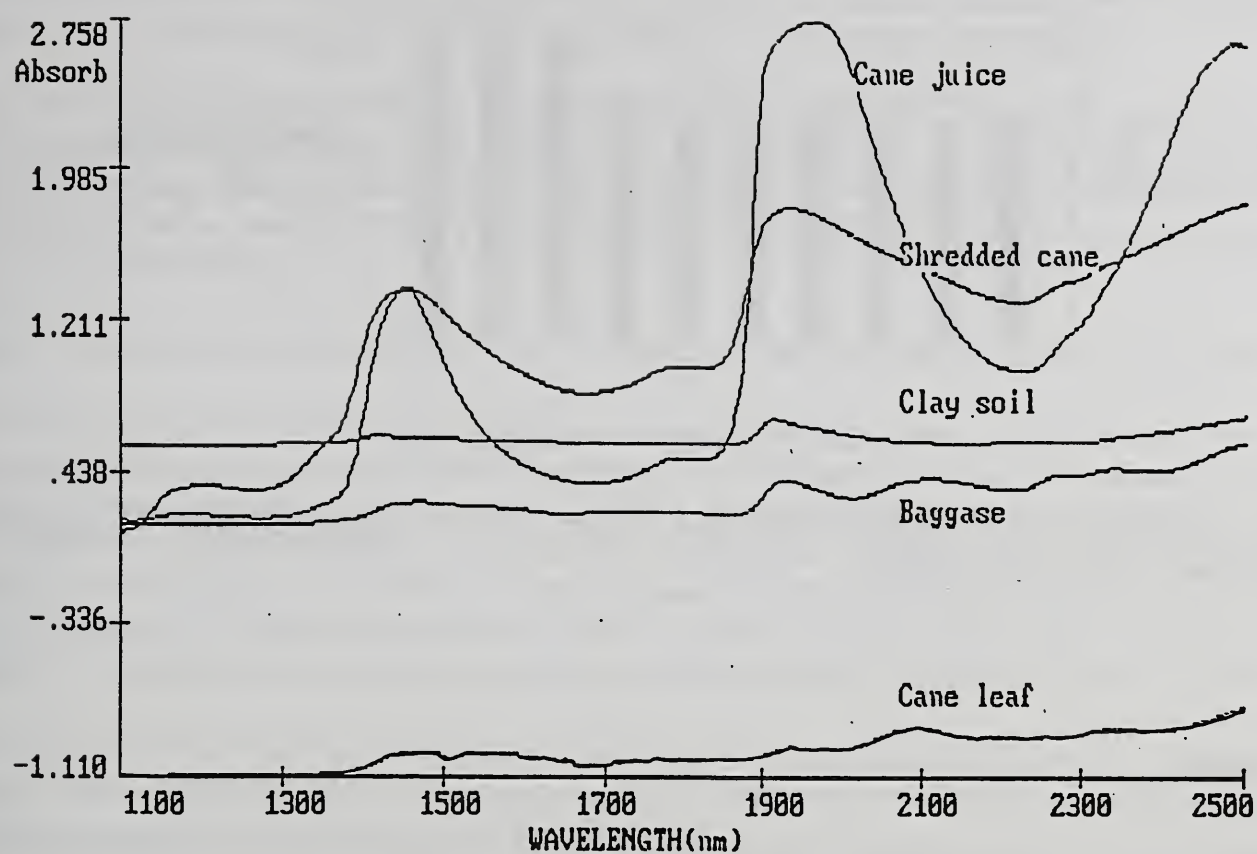


Figure 1. Comparison of typical NIR spectra for various products that have been researched at SASEX.

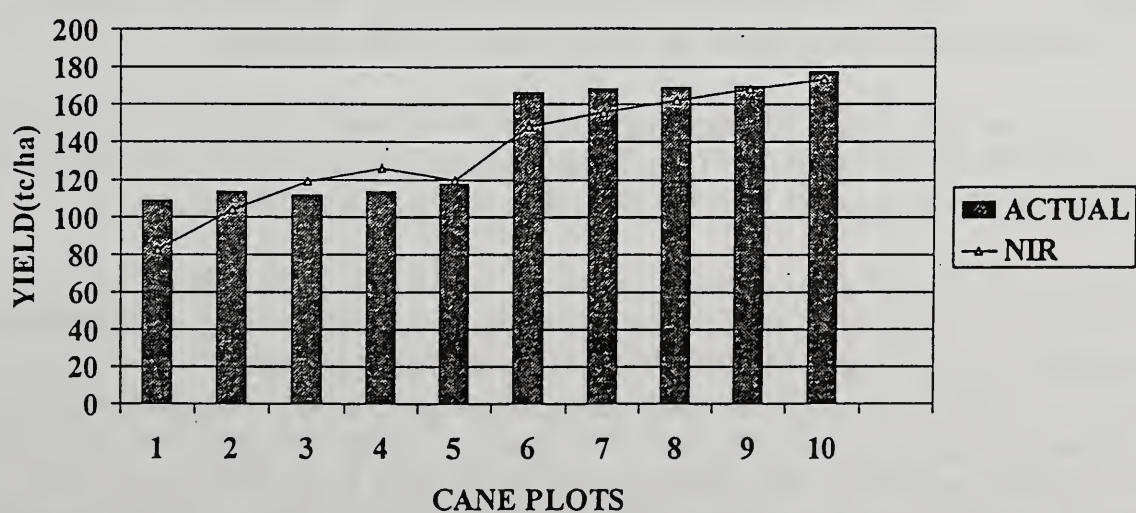


Figure 2. Actual cane yields obtained for variety CP66/14 (bars) plotted against predicted yields from NIR leaf scanning at 4 months.

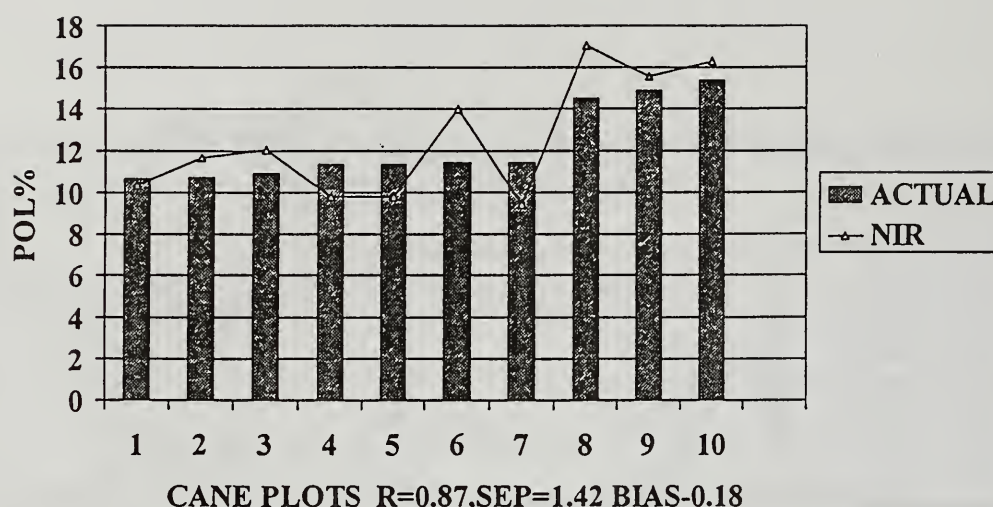


Figure 3. Actual pol % values obtained at harvest for variety NCo376 plotted against predicted pol % values from NIR lead scanning at 4 months.

DISCUSSION

Question: I am particularly interested in your analysis of leaves with regard to the preparation of the sample. Do you dry the leaves?

Meyer: Yes, we dry the leaves at 75°C overnight followed by grinding and pressing into discs. The advantage of discs is that they may be retained for many years and re-analyzed a number of times.

Question: Do you plan to convert to the Australian system of fibrating and analyzing shredded cane by NIR? Can you say a little more about the remote sensing NIR connection?

Meyer: If you are referring to Dr. Berding's NIR system based on an at line cassette sampler, it works extremely well but the throughput of 15 to 20 samples per hour is unfortunately too slow for our needs of routine analysis of cane samples from the Plant Breeding selection programme.

With regard to the second part of your question, spectral bands in the NIR region are increasingly being used for satellite remote sensing applications. However, the spectral range is usually restricted to the shorter wavelengths of the NIR region (850 to 1300 nm) and currently limited to four or five wavelengths. With most laboratory NIR scanning instruments the spectral range available is much wider (850 to 2450 nm) and up to 700 wavelengths are potentially available each of which coincide with the vibrational modes of different organic molecular configurations such as the N-H bond, C=O, O-H, etc. Laboratory NIR instruments should be used to calibrate remote sensing systems in terms of wavelengths associated with important biochemical processes in the crop canopy such as photosynthesis, chlorophyll production, the formation of carbohydrates and amino acids.

Question: Regarding the ethanol content of cane juice samples, were these samples taken from actual freeze damaged cane or was the build-up in ethanol concentration due to some other process?

Meyer: No, the samples were not taken from cane damaged by frost. The ethanol concentration in mixed cane juice samples is used by some mills as an index of cane deterioration brought about by delays between harvesting, stacking, loading and transport to the mill yard. Cane consignments containing high ethanol concentrations may be penalized. In our case freezing the shredded cane samples was used to prevent the samples from deteriorating.

Question: I am interested in the frequency of calibration for the products that you have described.

Meyer: This is certainly an important question, as a good calibration is the key to the successful use of NIR. We generally generate primary calibrations comprising 200 to 300 samples that show a normal distribution covering a representative range of the constituent values. For products such as shredded cane one needs to ensure that cane samples covering a wide range of geographic, seasonal and genetic differences are contained in the calibration. We follow the Dutch approach by checking calibrations on a weekly basis by scanning 10 samples of known composition. If bias is present for any product then we tend to add these samples to the calibration file and at the same time remove the values from the 10 oldest samples.

I also wish to stress the importance of using good primary data for calibrating instruments. With the direct analysis of cane, variations in the extraction efficiency of blenders or digestors in extracting juice from shredded cane can have a big influence on the calibration.

DEVELOPMENTS IN SUGARCANE AGRICULTURE THAT AFFECT PROCESSING

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ABSTRACT

Extensive research shows that sugarcane quality directly affects sugar yield and quality. Sugarcane with superior sucrose and purity and low fiber and trash content generally mills and processes well, producing a high yield of sucrose with few non-sucrose components. However, quality can be influenced by ever-changing developments in sugarcane agriculture including new cultivars, chemical ripeners, cultural practices and harvesting systems, and new disease and insect pests. These developments differentially affect the yield of sugar per unit area and cane and juice quality. Additional research has shown that these changing developments can have serious deleterious effects on the levels of Brix, sucrose, purity, fiber, reducing sugars, total polysaccharides including starch and dextran, phenolics, inorganic ash, proanthocyanidin, and other parameters of cane or juice. This paper will discuss how these developments in sugarcane agriculture affect processing.

INTRODUCTION

Through plant breeding, selection and, to a lesser extent, changes in cultural practices, sugarcane cultivars capable of producing high yields of sugar per unit area have been developed for both the tropics and subtropics (33). Concurrently, the yield of recoverable sugar per ton of cane has declined in many areas as the industries have switched to mechanized and green cane harvesting as opposed to hand harvesting of burned cane (31). As early as the 1940's, Keller and Seip (27) theorized that the major causes of this decline in Louisiana were four-fold: 1) adverse weather conditions; 2) reduced factory efficiency; 3) changes in cultivar preference; and, 4) mechanization of field operations. After a thorough investigation of each of these theories, they concluded that the decline in both factory efficiency and sugar recovery roughly paralleled the mechanization of the harvesting operations. While the extraneous matter in hand-cut, hand-loaded cane was less than 2.0% prior to 1942, it now generally exceeds 10% in machine-cut and -loaded cane. The Louisiana industry is experiencing yet another change in harvesting systems as it switches from the whole-stalk to combine technology primarily because of a new, high yielding cultivar that has a tendency to lodge. Further, as the cane is now harvested green, the extraneous material which includes cane tops, leafy trash, and field soil can exceed 20%. In the wet tropics of Australia, Paton (40) summarized work conducted by Drs. G.L. Wilson and J.K. Leslie who found that cane yields increased from 11 to 23% for Babinda and Mulgrave mill areas, respectively, from 1960 to 1995 with much of this increase coming from changes in cultivars. At the same time, sugar yields have decreased from 10 to 15% for the Mulgrave and Babinda mill areas, respectively. This difference in sugar yield was attributed to an increase in extraneous matter of about 1% in 1960 to 10% in 1995 delivered to the mill. According to Paton (40), this material is a major source of increased fiber, soluble impurities, and low sucrose content.

While cane and juice quality have, undoubtedly, suffered from this increase in extraneous matter delivered with cane for processing, the refineries and end users are demanding higher quality raws and refined sugars, respectively (6). Research has now been initiated by many of the world's sugarcane industries to assess the impact that new cultivars and harvesting systems are having on the efficiency of the milling process and the quality of the finished product (28, 40). Cane and sugar yield are still the major criteria used in the selection of new cultivars (18); however, more exhaustive research is now focused on evaluating the juice of new cultivars for other constituents besides Brix and sucrose content including concentration of reducing sugars and polysaccharides, especially starch and dextran, color and color precursors, and inorganic ash that can have a tremendous impact on processing and sugar quality. This paper will discuss the changes in sugarcane agriculture including cultivation of new cultivars, use of chemical ripeners, topping height, changes in cultural practices and harvesting systems, and the new disease and insect complexes that affect processing.

IMPACT OF CULTIVARS ON SELECTED QUALITY PARAMETERS

Starch. Research has shown that there are significant differences in the chemical composition of sugarcane (29, 36) and its parts (23). Starch is a polymer of glucose, found in many plants and existing in two morphological forms, namely, amylose and amylopectin. Starch in sugarcane is normally found at highest concentration in immature cane, the leaf blade and in the nodes (23). Starch becomes soluble in processing when juice temperature exceeds 71 °C. Rather than being eliminated in the mud during clarification, it remains in the clarified juice and concentrates in the syrup as the juice is evaporated where it increases viscosity and inhibits crystallization (23). The amylose form, in particular, slows the boiling rate, adding to energy usage, increases molasses volume with a corresponding loss of sugar, and is found in the raw sugar which can cause serious problems at the refinery. A study conducted by Godshall *et al.*, (19) showed that sugarcane cultivars differ significantly in the concentration of starch with concentration differences ranging as high as 5 times for the cultivars studied (Table 1). Further, it was determined that approximately 30% of the juice starch will end up in the raw sugar. Other studies (14, 21) have indicated that starch will cause process difficulties in the refinery when the concentration exceeds 200-250 ppm in the raw sugar.

Polysaccharides. Polysaccharides which can include starch and dextran as well as other large carbohydrate molecules (>10,000 MW) have several important functions in the sugarcane plant (19). However, polysaccharides are hard to remove in process and delay crystallization. Polysaccharides are found in the raw sugar and may contribute to color formation, thus adversely affecting sugar quality and increasing the cost of processing and refining. Godshall *et al.*, (19) also found significant concentration differences among cultivars with the range as high as two times for total polysaccharides (Table 1). Irvine (23) stated that polysaccharides are less abundant in the millable stalk than in the tops and leaves (green or dead) and stalk segments showed a decreasing concentration from top to bottom of the stalk.

Proanthocyanidins. Proanthocyanidins are related to plant pigments. The compounds turn red, pink, or orange under certain conditions, such as acidity, heat, and disease, and are associated with sugarcane polysaccharides (19). Sugarcane cultivars differ significantly in the concentration of proanthocyanidins in

juice as high as 6.5 times for the cultivars studied (Table 1). Proanthocyanidins are not yet associated with processing difficulties.

Phenolics. Phenolics are important from a processing standpoint because of their reactivity with metals and ability to produce highly-colored reaction products (17). As much as two-thirds of the color in cane juice may be due to enzymatic browning of phenolic acids (20). Phenolic compounds undergo non-enzymatic reactions including oxidation and self-polymerization into dark brown pigments. They react with proteins and amino acids to produce brown to black melanin pigments and with aldehydes to produce red condensation products in the presence of acids. Research has shown that the phenolic content of the colorant found in raw sugar can be used in conjunction with several other tests to indicate the ease of color removal of a raw sugar (8). Because of their ability to change color with pH, the presence of phenolic compounds in raw sugar can strongly affect color measurement. Godshall and Legendre (17) reported that there was a significant difference among cultivars in the concentration of phenolics in the juice as high as 2 times for the four cultivars studied (Table 2). In this study, phenolic content increased with maturity of the crop (data not shown). Further, phenolic constituents accounted for as much as 0.4% of refractometer solids of the juice of trash-free cane. However, as the level of trash increases with mechanically harvested cane, the concentration of phenolics would also increase, thus further exacerbating the problem.

USE OF CHEMICAL RIPENERS

Although plant breeding and selection of sugarcane have been responsible for the rapid increase in yield of recoverable sugar per unit area, sugarcane growers still depend largely on natural environmental conditions to trigger the maturation process. As an alternative to reliance on climatic factors affecting natural ripening, and with cognizance of the need of most growers to harvest immature sugarcane, research was begun on the use of bioregulators as chemical ripening agents (36). To date, hundreds of bioregulators have been evaluated world-wide for their ability to ripen sugarcane; however, the one most used is glyphosate (N-phosphonomethyl glycine). Nickell (38) stated that glyphosate formulations improve the sucrose content over a wide range of climatic conditions, are less cultivar-specific, and the ripening response induced in the sugarcane is more consistent and rapid than that obtained with most other compounds. The response to glyphosate is primarily in the top one-third of the sugarcane stalk (4). Because the growth retarding properties of the compound cause a reduction in overall cane yield, farmers using glyphosate may harvest cane without removing much, if any, of the top in order to maximize sugar yield.

The use of glyphosate does not come without potential adverse effects on processing, namely the decrease in reducing sugars-to-ash ratio and a possible increase in both total polysaccharides and dextran content which can ultimately contribute to sugar losses and lower sugar quality. Legendre, *et al.*, (36) noted that average total polysaccharides and dextran content (ppm on solids) of three cultivars as an average of five levels of topping was statistically higher for treated cane when compared to non-treated (no glyphosate) cane although there was a significant glyphosate*cultivar interaction (Table 3). However, no differences were noted for starch content between treated and non-treated cane. These results for dextran content were different to those found in a preliminary study conducted in 1985 (Legendre, B.L., USDA-ARS, SRRC, Houma, LA, unpublished data) as well as a follow-up study conducted in 1986, where no difference was

found in dextran content (ppm on solids) in cane harvested 56 days after treatment (Legendre, B.L., USDA-ARS, SRRC, Houma, LA, unpublished data). In both of these preliminary studies the concentration of dextran (ppm on juice) was numerically higher but not significantly different for treated cane when compared to non-treated cane. Further, by not removing the top portion of the stalk in treated or non-treated cane, there are other potential adverse effects such as increased reducing sugars, inorganic ash, and starch content which all affect processing. The effect of topping height on processing will be explained in more detail in the next section.

TOPPING HEIGHT

It has been recognized since 1935 that topping height has considerable effect upon the quality of juice from harvested sugarcane (2). Mechanical harvesting with the whole-stalk or "soldier harvester" led to the concept of "average topping" rather than the older practice of topping each stalk at the uppermost mature internode when cane was cut by hand (9). However, today in heavy lodged cane little, if any, topping is practiced when cane is harvested by combine. When sugarcane stalks were topped too low by the whole-stalk harvester, mature cane on the taller stalks was left in the field resulting in a loss of cane yield. However, when the stalks were topped too high, immature cane on the shorter stalks would be delivered to the mill with a resulting loss of sugar yield (11). This relationship is most important in immature cane.

Proper setting of the topping height by the harvester operator has an important effect on the quality of harvested sugarcane. It was recognized in Louisiana as early as the 1940s that mechanical harvesting resulted in increased cane trash, including tops. This decreased the level of both normal juice sucrose and purity. Further, milling of cane with tops reduced the available sucrose and increased the amount of non-sucrose solids, resulting in a loss of recoverable sugar and an increase in molasses volume.

Numerous investigators (32, 34, 36) have studied the effects of tops on processing. Both sucrose and purity % cane increased with the severity of topping while reducing sugars were highest in cane with intact tops. The concentration of reducing sugars decreased with the severity of topping. Significant differences were also noted in inorganic ash % total solids with the severity of topping. There was higher inorganic ash % total solids in extracted juice with no topping; the level of ash was significantly lowered by removing the tops (Table 4). Further, there were significant differences in the ash content among cultivars. High ash content is associated with lower boiling house efficiency and recovery and contributes to a higher volume of final molasses with a significant loss of sugar. In certain production areas such as south Texas, concentration of ash in extracted juice is used as a selection criterion in cultivar development. Legendre *et al.*, (36) noted that there were significant differences in the concentration of ash among cultivars regardless of the severity of topping. Higher topping also contributed to higher total polysaccharides, starch, and proanthocyanidins in the extracted juice.

CHANGES IN HARVEST SYSTEMS

According to de Beer (12), reduced labor availability and very small profit margins mandated mechanization of the sugarcane handling process. Recently, de Beer and Purchase (13) stated that it is unfortunate that as

field operations are mechanized, field losses increase and the quality of the product delivered to the mill for processing decreases. For both manual and mechanical harvesting of sugarcane, the condition of the harvested crop has a significant effect on quality of cane delivered to the factory (28). Typical quality factors include the maturity of the crop, quantity of tops and leafy trash, number of suckers, dead cane, roots, and soil.

Green cane harvesting. Recently, de Beer and Purchase (13) listed the advantages of green cane harvesting on raw factory operations. They are as follows: 1) greatly reduced dextran levels in juice; 2) stale cane reduced to a minimum with the onset of rainy weather that may delay or stop the harvest entirely; and, 3) greater overall recovery of sugar per unit area of land. However, green cane harvesting also has its disadvantages. They are: 1) more extraneous matter in harvested cane; 2) greater concentration of waxes, ash, and starch in extracted juice, increased molasses volume, increased sugar losses per ton of cane, and reduced milling capacity resulting in increased cost per unit of sugar produced; 3) more waxes entering the factory; and, 4) a higher concentration of color in juice and raw sugar reducing its value. Of course, many of these constituents will carry over into the refinery causing similar problems. They further stated that adverse agronomic practices cannot usually be completely offset by improved harvester design or harvesting practices and it is important that agronomic practices be closely aligned to harvesting requirements. Although the combine can harvest green cane, many growers are burning standing cane prior to harvest to increase the efficiency of the harvester, which also results in less trash.

Trash. Paton (40) summarized results of research conducted in Australia which found that the switch to mechanized and green cane harvesting as opposed to hand harvesting of burnt cane was responsible for the increased trash (extraneous matter) in cane delivered to the factory resulting in lower sugar yields. He also noted that new, high yielding cultivars have a greater tendency to lodge and produce an abundance of "water shoots" or suckers which contribute to the increased extraneous matter. Another factor to consider is the use of increased fertilizer inputs with the new cultivars that further contribute to lodging. Many growers in Louisiana have now switched to the cane combine to harvest newer cultivars that produce higher yield but are prone to lodging; however, the amount of harvested trash in cane is significantly higher when compared with cane harvested by hand and topped between the 3rd and 4th visible dewlap to simulate cane cut by the whole-stalk harvester (35) (Table 5). The combine chops the cane stalks into billets of 17.5 to 35.0 cm and, with proper operation, can remove a significant portion of the leafy trash without burning. On the other hand, little or no topping is practiced in lodged cane. Harvesting cane under wet field conditions generally result in a higher level of extraneous material, including mud, delivered to the mill for processing.

Deterioration. Research has also shown that burnt and/or chopped cane deteriorates faster than whole-stalk cane (Table 6) and should be processed within 20 hours of harvest because of the formation of dextran produced by the bacterium, *Leuconostoc mesenteroides* (24, 25, 35). The presence of dextran indicates specific loss of sucrose. Clarke (7) stated that the major factors affecting dextran levels in raw sugar are related to field and harvest conditions. These include: 1) ambient temperature; 2) ambient humidity; 3) rainfall and mud; 4) degree of burn of cane; 5) integrity of the cane stalk or billet (degree of damage); 6) billet or stalk length; 7) delay between burning and cutting; and 8) delay between cutting and crushing. With

increasing time, dextran levels increase asymptotically, while with increasing temperature and humidity, dextran concentration of the juice increases dramatically. Sugarcane cultivars may also have an effect on susceptibility to *Leuconostoc* infection while in the temperate areas where sugarcane is grown, freeze damage can also have a strong influence on the concentration of dextran.

The best control for dextran is to inhibit its formation. A major aspect of control is the coordination of harvest schedules with factory deliveries and crushing. Good coordination of harvest and delivery with minimum holding times after burn or harvest, are important for all cane, but especially for combine-harvested or billeted cane, with its greater amount of exposed tissue (30). Dextran can be formed at the factory, first in the cane yard, and second, in juices and waters containing sucrose all across the mill tandem until juices are heated. Control of *Leuconostoc* and dextran production in the mill is best accomplished by good hygiene, good housekeeping, and the judicious use of bactericides. However, if dextran is already present in the juice because of poor quality cane or mill hygiene then control may be accomplished by either treatment by dextranase enzyme or removal by syrup clarification. Dextran causes numerous problems in processing including increased viscosity, boiling time and energy use, elongation of the sucrose crystal along the C-axis, and, most importantly, loss of sugar with increased molasses volume in both the raw cane sugar factory and the refinery (3, 8).

Color formation. With the changes in harvest systems comes increased extraneous matter. In South Africa (5), it was reported that with each 1% addition of tops to clean cane the color of clear juice as measured by absorbance was increased by 1.3% while with each 1% addition of mud to clean cane the color of clear juice was increased by 3.6%. Subsequent studies by Purchase *et al.*, (41) in South Africa showed that trash contributed substantial color and turbidity to juice. He found that trash appeared to contribute more color than tops alone but when results were normalized there was a linear relationship between color in juice and extraneous material.

In Australia, Ivin and Doyle (26) defined trash as incorporation of the remains of leaves attached to the cane stalk but not including field soil. Further, tops were defined as that portion of the cane stalk above the break point approximately 25 cm in length, minus the top leaves normally cut and blown clear by the cut-chop (combine) harvester. They found that juice color increased an average 25% with the addition of 6% trash (i.e., a 4.2% color increase for every 1% trash). They also noted a 12% increase in juice color by the addition of 6% green tops (i.e., a 2% increase for every 1% tops); however, the increases in juice color were highly dependent on the cultivar of cane as well, with tops contributing from as little as 2% color in one cultivar and as high as 29% color in another. The range for trash effects alone on color by cultivar was from a low of 9.8% to as high as 47.6%.

Legendre *et al.*, (37) investigated the effect of leafy trash on color in Louisiana. They found more than a 6-fold increase in color over the treatment range studied (Table 7). There was a 13% increase in juice color for every 1% leafy trash added, up to the 10% level, which was within the range of the Australian experience (26). Field soil (mud) alone showed a decolorizing effect, due, undoubtedly, to the ion exchange properties of the soil type (heavy clay)(37). The effect of mud was linear with an approximately 1.6% decrease in color per 1% of added mud up to the 30% level. These results were contrary to the results found in South Africa where mud also increased the color of juice (5). The results for combination effects of leafy cane trash and

mud mixture reflect the opposing effects of the two components (37). These results further show that the components of trash can have different effects on cane juice color, and it is important to define the composition of the trash.

DISEASE AND INSECT COMPLEXES

Plant phenolic concentration has been correlated with fungal, bacterial, and insect resistance (10, 16). Many fungal diseases of sugar cane, notably red rot, but also *Fusarium* wilts and rots, *Helminthosporium* eye spot, and others, induce the sugar cane plant to produce red phenolic pigments (15). It is possible that an increase in phenolic concentration may also be a general stress response caused by mechanical and chemical injury, sugarcane borer damage, and bacterial and viral disease-induced tissue reddening.

Yellow leaf syndrome. It has been suggested that a relatively new disease, yellow leaf syndrome (YLS), caused by a virus, can result in an increased concentration of starch in juice; however, reports obtained from Brazil (Ulian, E., Copersucar, personal communication) indicated that an analysis of juice of stalks of the cultivar SP 71-6163 for different carbohydrates, including starch, comparing severely affected plants with plants with no symptoms, showed no significant differences. This was not the case for juice extracted from leaf tissue, especially from the midribs, where there was a large increase in both starch and sucrose in plants infected with YLS when compared to plants showing no symptoms. Preliminary studies conducted in 1997 in Louisiana (Grisham, M.P., USDA-ARS, SRRC, Houma, LA, unpublished data) confirmed this phenomenon as juice from the tops of sugarcane plants (cultivar LCP 82-89) infected with YLS had a significantly higher concentration of Brix, sucrose, and purity as well as a higher estimated yield of theoretical recoverable sugar per ton of cane when compared to healthy plants (Table 8). However, in these studies, no differences were found for any of these parameters in the juice extracted from clean stalks between diseased and healthy plants. Although no analysis was performed for starch, it may be assumed that infected plants also contained higher concentrations of starch as well. Accordingly, it also may be safe to assume that the concentration of starch will increase in harvested cane infected with YLS because of the increase in the extraneous matter, including tops, delivered to the mill for processing as a result of mechanical harvesting.

Leaf scald. In four field studies conducted in Louisiana, Hoy and Grisham (22) stated that a potentially serious bacterial disease, leaf scald, caused by *Xanthomonas albilineans*, reduced the concentration of sucrose and purity in extracted juice of symptomatic stalks when compared to asymptomatic stalks. In India, Agnihotri (1), reported that leaf scald also caused a 2.2 to 9.5 fold increase in reducing sugars in the extracted juice of two major cultivars, BO 70 and Co 62399, respectively, as well as lowering the concentration of both sucrose and purity. In addition to affecting cane yield, leaf scald infection also affects the quality of juice by lowering Brix, pol, and purity. The extent of the losses being directly related to the level of infection (42). According to Osgood and Teshima (39), the presence of reducing sugars decreases juice purity which may ultimately have an adverse effect on sucrose recovery.

SUMMARY AND CONCLUSION

This paper has presented a general description of the changes in sugarcane agriculture that affect processing, including new cultivars, chemical ripeners, topping height, cultural practices, harvest systems, and new disease and insect pests. However, there is still much research required to fully qualify and quantify the impact that these agricultural operations have on processing. In summary, production and harvest management are key to optimizing cane quality, and production, harvest- and post-harvest management are key to juice and sugar quality.

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Table 1. Starch, total polysaccharides and proanthocyanidins concentration of ten sugarcane varieties as an average of four harvest dates ^{1, 2}.

Cultivar	Starch concentration (ppm on solids)	Total polysaccharides (ppm on solids)	Proanthocyanidins (A ₄₈₅ x 1000)
CP 65-357	506 DE	2,128 CD	40 C
CP 70-321	275 F	1,455 F	15 H
CP 72-356	733 C	2,469 B	34 D
CP 72-370	1,460 A	3,234 A	65 A
CP 74-383	611 CD	1,931 DE	10 I
CP 76-331	647 CD	1,876 E	59 B
CP 79-318	986 B	2,577 B	20 G
CP 82-551	450 E	1,657 F	31 DE
LCP 82-89	566 DE	2,161 C	22 FG
Nco 310	423 E	1,901 E	27 EF
Mean	275	2,139	32

¹ Means followed by the same letter in columns are not significantly different at the 95% confidence level.

² Godshall *et al.*, Int. Sugar J. 98(1168):144-148. 1996.

Table 2. Phenolic concentration of five sugarcane varieties as an average of four harvest dates and two methods of milling (roller and press)^{1, 2}.

Cultivar	Mean caffeic acid equivalents (µg/m juice)
CP 65-357	686 A
CP 70-321	393 D
CP 74-383	542 B
Nco 310	499 C
Mean	530

¹ Means followed by the same letter in column are not significantly different at the 95% confidence level.

² Godshall and Legendre, Int. Sugar J. 90(1069):16-19. 1988.

Table 3. Glyphosate treatment on total polysaccharides and dextran concentration (ppm on solids) of three cultivars as an average of five topping heights ^{1, 2, 3}.

Cultivar	Non-treated (no glyphosate)	Glyphosate treated
------(ppm on solids)-----		
<u>Total polysaccharides</u>		
CP 65-357	4,855 a	4,661 a
CP 70-321	3,718 a	3,840 a
CP 72-370	6,103 b	11,790 b
Mean	4,892 A	6,740 B
<u>Dextran</u>		
CP 65-357	126 a	512 b
CP 70-321	235 ab	105 a
CP 72-370	418 b	1,272 c
Mean	306 A	639 B

¹ Means followed by the same letter in rows (upper case), read across, and in columns (lower case), read down, are not significantly different at the 95% confidence level.

² Legendre *et al.*, ACS Symposium Series 557, pp 26-38. 1994.

³ Five topping heights = no topping and 5 cm above and 10, 25 and 40 cm below apical meristem (bud), respectively.

Table 4. Effect of topping height on the concentration of inorganic ash % total solids in extracted juice of three cultivars ^{1,2}.

Cultivar	Topping height (level of topping) ³					Mean
	1	2	3	4	5	
	------(Inorganic ash % total solids)-----					
CP 65-357	0.56 b	0.54 ab	0.45 ab	0.42 ab	0.41 a	0.48 B
CP 70-321	0.63 b	0.52 ab	0.52 ab	0.48 a	0.46 a	0.52 B
CP 72-370	0.48 b	0.42 ab	0.37 ab	0.37 ab	0.34 a	0.40 A
Mean	0.56 c	0.49 bc	0.45 ab	0.42 ab	0.40 a	0.47

¹ Means followed by the same letter in rows (lower case), read across, and in columns (upper case), read down, are not significantly different at the 95% confidence level.

² Legendre *et al.*, ACS Symposium Series 557, pp 26-38. 1994.

³ 1 = no topping; 2, 3, 4 and 5 = 5 cm above and 10, 25 and 40 cm below apical meristem (bud), respectively.

Table 5. Trash content of green and burnt sugarcane (cultivar LCP 85-384) harvested by hand and combine at different extractor fan speeds ¹.

Harvest system	Fan speed	Trash content	
		Condition of harvest	
		Green	Burnt
		------(%)-----	
Combine	Fast (1,200 RPM)	11.5	8.2
	Med. (1,020 RPM)	15.8	11.6
	Slow (240 RPM)	17.2	10.2
Hand-cut ²	Not applicable	10.9	5.8

¹ Legendre and Richard, Sugar y Azucar 92(6):35-36. 1997.

² Trash content of hand-cut cane based on stalks topped between 3rd and 4th visible dewlap.

Table 6. Effect of burning and harvest system on the deterioration of sugarcane (cultivar, CP 70-321) measured by the concentration of dextran in extracted juice ^{1,2,3}.

Treatment ³	Dextran concentration (ppm on Brix)	
	Whole-stalk	Combine
Unburned stalks -		
Control	64 A	95 A
1 DAH	109 A	1,850 B
2 DAH	312 A	3,423 D
Burned stalks -		
Control	153 A	253 A
1 DAH	166 A	2,968 C
2 DAH	276 A	3,155 CD

¹ Means followed by the same letter in columns are not significantly different at the 95% confidence level.

² Legendre and Richard, Sugar y Azucar 92(6):35-36. 1997.

³ DAH = days after harvest.

Table 7. Average values for ICUMSA color of centrifuged cane juice (cultivar CP 70-321) determined at 420 nm in the presence of various admixtures of leafy trash (LT) and field soil (FS) (mud) ¹.

Admixtures	Trash content	ICUMSA color
	%	(420 nm)
Clean cane	0	2910
LT	10	6,665
	20	12,148
	30	19,432
FS	10	2,554
	20	1,799
	30	1,315
LT + FS	5 + 5	6,208
	10 + 10	7,084
	15 + 15	7,645

¹ Legendre *et al.*, Proc. Conf. Sugar Proc. Res., pp. 447-452. 1996.

Table 8. Brix, sucrose and purity of juice from sugarcane tops (cultivar, LCP 82-89) infected with yellow leaf syndrome (+YLS) compared to plants free of the disease (-YLS) ¹.

Components	Yellow leaf syndrome	
	Positive (+)	Negative (-)
	------(%)-----	
Brix	8.7	7.6*
Sucrose	12.4	8.9*
Purity	36.0	29.4*

¹ Means in each row for (-) followed by * are significantly different from means for (+) at the 95% confidence level.

DISCUSSION

Question: Researchers from S.P.R.I., South Africa and Australia express dextran concentration, as you do, as ppm on Brix. What is the best way to express the amount of dextran in juice and in cane?

Legendre: In most cases, we also express dextran on soluble solids or Brix. We know that many of the things we do in agriculture will actually increase the Brix or sucrose of the product that is being delivered to the mill. In some of the processes, we can actually increase the level of dextran in the juice, but when the concentration of dextran is expressed on a soluble solids basis (Brix), the actual concentration of dextran based on the soluble solids actually goes down. Accordingly, the overall concentration of dextran and the effect of dextran on processing is negligible.

Question: With regard to the combine and green cane, there is a trend that eventually most of the cane will be harvested green. In Columbia's Valle del Cauca, by the year 2000, everything has to be green. In certain areas of Florida, they cannot burn the cane when the wind is blowing a certain direction.

You were talking about the trash that comes to the mills which is running about 15-20%. In your experience, what happens to the field because the amount of material that stays on the field creates two problems. One problem is, how do you adjust the base cutter to know where you are cutting? You have no idea where you are cutting, especially if you are harvesting at night like they do in Columbia. The second problem is what happens to the cane in there because basically you can get a lot of deterioration at the roots of the cane plant because you are not getting enough sun.

Legendre: We have found, as they have in Australia where they have harvested green cane for quite some time, (we have only harvested green cane in Louisiana for four years and we are now studying the effect of the trash blanket), that when you adjust the fan speeds of the harvester from 240 to 1200 rpm in an attempt to remove trash, you place more residue on the ground behind the harvester, e.g. from less than 5 tons to more than 15 tons, respectively. If the extractor fan speed is reduced, you are actually delivering anywhere from 10-15 extra tons of non millable cane material to the mill. Most of that is trash. If you leave it on the field, we find, under Louisiana conditions, you can actually reduce the yield of cane in the subsequent stubble crop by as much as 5 tons of cane per hectare. Therefore, we recommend removing the trash blanket in some way; otherwise, you risk the yield of the stubble crop. You can take the residue off by raking, shaving or (if all else fails) by burning.

Regarding the base cutter, there are controls on the combine that indicate changes in hydraulic pressure upon contact with the ground. Adjustments are then made to limit contact with the ground to reduce the amount of mud (field soil) in the harvested cane. However, by the time the adjustment is made it is usually too late and several hundred kilos of mud can be found in harvested cane.

To prevent mud in deliveries it is recommended that the base cutter be set at 8-12 cm above the ground level, especially under wet field conditions.

Question: As a plant breeder, to what extent will the results of your investigations influence the selection of varieties?

Legendre: At the present time, my basic philosophy is to select varieties based on total recoverable sugar per hectare. However, we will use the results of these investigations in the selection of parents to be used in producing the next generation of varieties. To cross varieties high in starch with another variety high in starch would lead to unacceptable levels of starch in the progeny.

Question: What is the rate of biodegradation of the trash blanket on the field during the course of the season, and what is the fate of that material in the soil?

Legendre: Our conditions are quite wet during our winter months. Last year, in January, following the end of our harvesting season, we had over 50 cm of rain. Soil moisture remained at 100%. We then had a very cool spring with relatively little biodegradation during that period of time, especially under the trash blanket. The trash blanket kept the soil wet, and a lot of the stubble rotted in the soil. Tests to date have shown that leaving this trash blanket in place can result in a loss of as much as 5 tons of cane per hectare in the subsequent ratoon (stubble) crop. However, when dry, the effect is not so dramatic. Typically, the cane will grow through it and the trash blanket can actually contribute to weed control.

Research is underway with the use of various bacterial concoctions on the trash blanket to try to speed up degradation, even under cold temperatures and high humidity. It seems to help; however, the recommendation is still to remove the trash blanket and, the sooner after harvest the better.

A SOUTH AFRICAN PERSPECTIVE ON OLIGOSACCHARIDES AND CANE SUGAR PROCESSING

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ABSTRACT

An overview of recent experiences in analysing and monitoring oligosaccharides is presented. High performance anion exchange chromatography (HPAEC) with electrochemical detection has been used extensively to obtain typical 'oligosaccharide' profiles. Analytical advantages and disadvantages are discussed.

Oligosaccharides have been found to be the principal cause of crystal elongation in the South African sugar industry. Five major oligosaccharides have been identified in process. It has been demonstrated that one of these (theandrose) promotes c-axis elongation, but it is clearly not the only factor involved. This sugar has been detected in mixed juice, is strongly transferred to the crystal and can be used to differentiate cane and beet crystals.

The accumulation of oligosaccharides between harvesting and crushing of whole stalk cane has been monitored. Pilot plant studies have been used in an attempt to highlight how the presence of these sugars may impact on aspects of cane processing and sugar quality.

INTRODUCTION

A program to examine the causes of crystal elongation was introduced at the SMRI some years ago. Investigations indicated that oligosaccharides were a major contributor to habit modification and steps were taken to identify these oligosaccharides and to establish their origin (9,10,11). In subsequent work, attempts have been made to relate the impact oligosaccharides might have on processing and the effect of processing on these oligosaccharides (13). The development of suitable analytical techniques played an important part in these investigations. These studies are reviewed in this paper and some of the findings are highlighted.

EXPERIMENTAL

The experimental procedures have been published elsewhere -- laboratory crystallisation techniques and preparative isolation procedures have been described (9), analytical methods have been discussed (10), and HPAEC conditions and calibration procedures have been documented (13,16). The operation of the pilot plant was described by Lionnet and Reid (7).

RESULTS AND DISCUSSION

Oligosaccharides and crystal habit

Crystal elongation describes the shape of a crystal when the ratio of the crystal lengths in any two specified directions is different from that usually observed under similar crystallisation conditions. In the cane sugar industry the c/b ratio is often used. Sucrose grown in pure aqueous solution is approximately twice as long in the b -axis direction as it is in that of the c -axis i.e. any sucrose crystal with a c/b ratio greater than 0.5 is elongated in the direction of the c -axis. The greater this ratio, the greater the extent of elongation. The general outward appearance of the crystal is governed by conditions during growth which can affect the growth rates of the different crystal faces relative to each other. Several factors can influence these growth rates -- both physical and chemical, e.g. temperature, viscosity and type and concentration of impurities.

In South Africa sucrose is produced exclusively from sugarcane. Some degree of crystal elongation is nearly always observed in both refineries and raw sugar factories. The effect is usually accompanied by lower crystallisation rates. Generally, the effect is enhanced at the start and, particularly, at the end of the milling season which runs from about April or May and continues until January or sometimes February. Typical shape ratios for factory C-masseccuite are shown in Figure 1. In general, lower purity refinery products tend to give the most elongated crystals with elongation (and pan boiling times) increasing quite dramatically as the masseccuite purity drops.

A reproducible laboratory-scale crystallisation technique was used to grow crystals at temperatures and saturations similar to those encountered in the factory and in the presence or absence of selected impurities. The limited amounts of isolated impurities dictated the laboratory scale crystallisation procedure. Using this technique it has been shown that most of the crystal elongation encountered in cane processing in South Africa can be attributed to the presence of oligosaccharides, although no single component is totally responsible. The sucrose/water (S/W) ratio was kept constant at 3.075 (supersaturation of 1.06 for pure sucrose), temperature at 60.5°C. The non-sucrose/water (NS/W) ratio varied from 0.05 to 0.60. Rotation speed was constant. Sucrose crystal shape was independent of size within the range of crystal sizes encountered and elongation increased as the impurity concentration increased (9). Since the physical conditions were controlled it was concluded that the concentration of habit modifying impurities (i.e. the quality of the impurities) exerted the overriding influence on the crystal shape. In subsequent comparisons the NS/W ratio was held at 0.1 (97 purity) for refinery products and at 0.5 (86 purity) for factory products to enable the effects of different impurities to be evaluated. This usually gave a c/b ratio of about 1.35 as the reference point.

Molasses was classified into high and low molecular mass fractions using selective ethanol precipitation and was further sub-divided using carbon chromatography and elution with increasing ethanol concentrations. Evaluation of the effects of the different fractions demonstrated that both the shape modifying and rate retarding components were in the oligosaccharide fraction. Re-combination of the fractions showed that there had been no significant loss of any elongating properties (Table 1). The habit modifying properties of the oligosaccharide fraction were completely removed after invertase hydrolysis. Normal shaped crystals were obtained when sucrose solutions were spiked with the hydrolysate. A similarly buffered control showed minimal improvement in elongation (Table 2). This implies that the elongation promoting factors are

fructose containing oligosaccharides, probably sucrose derivatives. Smythe (17) found that particularly potent modifiers were those oligosaccharides in which a hexose was substituted on carbon 6 of the glucose moiety of sucrose e.g. raffinose, neo-kestose. Theandrose, subsequently identified as a typical impurity of cane sugar (11), would be included in this group.

Furthermore, the oligosaccharide mixtures obtained from fractionation on carbon columns have been scanned using Fractogel TSK HW 40 (S). The HMWt fraction (which was found not to affect crystal habit) was not retained (i.e. no significant concentration of compounds of molecular weight less than 7,000 was found in this fraction). The fraction causing habit modification appeared to contain only tri- and tetrasaccharides.

South African raw sugars are generally c-axis elongated, but usually show negligible dextran levels using the haze technique (4). Dextran (AOAC) levels exhibit a pronounced seasonal trend, with most of the 'dextran' being medium M Wt, i.e. precipitated with 80% ethanol, but not with 50% ethanol. Typical results are shown in Table 3 where, although dextran ranged from 100 to 300 ppm, the high M Wt fraction was less than 90 ppm. On several occasions it was found that enzymic removal of dextran in refinery or laboratory trials led to improved performance, but gave no change in crystal shape (Table 4). In addition, comparison of the shapes of crystals grown in laboratory massecuites prepared from the high and low M Wt fractions from mid and end season molasses samples, confirmed that despite the obvious adverse effects of dextrans on processing, the presence of oligosaccharides had a more significant effect on crystal habit (Table 5). The oligosaccharide level increased three-fold during this period.

Hidi and Staker (6) demonstrated that significant enzymic dextran removal under factory trial conditions was accompanied by insignificant improvement in crystal elongation e.g. decreasing dextran from 1700 ppm to less than 100 ppm in incoming juice only decreased the c/b ratio from 1.33 to 1.11 and decreasing dextran in C-masseccuite from 4300 ppm to about 1720 ppm only improved the elongation from 2.22 to 2.0. Tilbury (19) could find no statistically significant correlation between dextran content and crystal elongation in C-masseccuite during a period when high dextran levels were entering the factory although all samples showed marked crystal elongation. Mantovani, *et al.*, (8) have also indicated that the contributions of glucose, fructose and dextran to sucrose crystal morphology are probably due to a competitive mechanism and are less important than other major elongating effects observed in industry. Despite the evidence that sucrose crystal elongation is not directly attributable to dextran, the other processing problems associated with polysaccharides or dextran should not be underestimated. Such processing problems will directly affect throughput and exhaustion.

These early trials implicated oligosaccharides in crystal elongation. Hence, further investigations focussed on establishing the source of these oligosaccharides and their contribution to processing problems. Reliable analytical methods were developed for this purpose.

Oligosaccharides and analysis

Alcohol precipitation, classical carbon-celite chromatography and SEC (Fractogel HW 40) were used in the early stages of investigations at the SMRI to obtain crude separations of components from industrial streams

(e.g. refinery or factory molasses). Great reliance was placed on thin layer chromatography for monitoring and semi-quantitation. Although these procedures were tedious and cumbersome they did enable the collection of sizeable fractions (all mixtures) so that the effects of different components could be evaluated (9).

A number of analytical techniques were tried, none of which lent themselves to examining large numbers of samples and all of which had some drawbacks. These included small scale carbon-celite enrichment (poor recovery of early eluters), thin layer chromatography (semi-quantitative and subjective), gas chromatography (tedious derivatisation) and C-18 HPLC (poor column life). The introduction of HPAEC opened the way to more intensive monitoring and it has become the method of choice for carbohydrate analysis.

Anion exchange chromatography has been used extensively for a variety of sugar separations. When coupled with pulsed amperometric detection (PAD) it has proved to be a powerful tool for the analysis of closely related oligosaccharides. Changes in solvent composition (and to some extent column temperature) allow extraordinary flexibility in the range of sugars which can be separated. The pellicular nature of modern anion exchange phases allows rapid solute-solvent equilibration, but limits column loading. However, low concentrations of sugars are readily detected with the high sensitivity of PAD and the CarboPac columns tolerate a fair degree of overload from sugars which are not being quantified. The high purchase price of these columns is offset by their ruggedness and long life. The sugars behave as weak acids and the resolution between peaks is governed by the pKa of the particular compounds. Maximum resolution is generally obtained when the solvent pH is close to the pKa of the compound of interest. At pH values above the pKa, retention times decrease without further improvement in resolution and often with changes in elution order for different families of oligosaccharides. Because relatively few pKa values for carbohydrates have been reported, chromatographic separations are largely based on trial and error techniques. A combination of pH adjustment (by means of sodium hydroxide concentration and column temperature) and the addition of salts, such as sodium acetate or nitrate, leads to the resolution of specific peaks within an acceptable analysis time. Sodium hydroxide, in the range 100 to 150 mM is usually used. At sodium hydroxide concentrations below 100 mM, problems with carbonate build-up on the column can be expected. The solvent composition of 100 mM sodium hydroxide (NaOH)/15 mM sodium acetate (NaOAc) has been used routinely at the SMRI for the isocratic separation of the major cane oligosaccharides in less than 30 minutes. A typical separation of reference sugars is shown in Figure 2. However, because of the nature of anion exchange chromatography this solvent can only be regarded as a compromise. It is sometimes necessary to adjust the solvent for specific applications. The separation of raffinose, stachyose, maltose and isomaltotriose (common in seed extracts) provides a good example. Raffinose and stachyose co-eluted when the usual solvent was used. Maximum resolution of raffinose and stachyose was evident at sodium hydroxide concentrations greater than 125mM. However, isomaltotriose was only resolved from raffinose at sodium hydroxide concentrations greater than 150 mM. Maltose co-eluted with stachyose at sodium hydroxide concentrations above 160 mM. Hence a solvent composition of 150 mM NaOH/10 mM NaOAc was selected for this particular application. Although acceptable separations of cane oligosaccharides are obtained with the solvent combination 100 mM NaOH/ 15 mM NaOAc, the separation of raffinose, stachyose, maltose and isomaltotriose is a good illustration of how the system can be better optimised for specific applications (Figure 3). It can also be seen from Figure 2 that different oligosaccharide families (e.g.

the malto- and isomalto-oligosaccharides) elute at different rates so that retention time in general gives no indication of molecular size, unless an homologous series is separated.

In addition to factors affecting separation efficiency, several factors can influence the sensitivity of amperometric detection. The amperometric detector response is a rate-dependent electrochemical reaction and detector response is temperature dependent, with different sugars behaving differently to temperature variations. Some detector selectivity can also be achieved by adjusting the measuring potential (E1). For example additional PAD responsive components can be detected in mixed juice if E1 is set too high, e. g. 200 mV. By selecting a lower applied potential for E1 (50 mV), detector specificity towards carbohydrates can be improved (16).

Although the separating efficiency of CarboPac columns is remarkable, baseline separation is seldom achieved, except for simple mixtures. Thus peak height is preferred to peak area for integration. Different oligosaccharides show quite variable response factors. Nevertheless, raffinose (which is readily available commercially) has been used for calibration after adjustment for retention time influences on the area/height ratio (13). Unless authentic oligosaccharides are available, such a compromise is necessary. Partial resolution also means that closely eluting peaks become merged when the concentration of either is much greater than that of the other. This occurs frequently with the 1-kestose-theandrose doublet, the latter usually being seen as a shoulder on the 1-kestose peak -- a further reason for choosing peak height.

HPAEC has many advantages -- it is quick, simple and useful when handling large numbers of samples. That compounds other than sugars can be detected and that response is different for different sugars is a minor drawback. As with most HPLC techniques, limited separation efficiency causes detection problems when there are large concentration differences between closely eluting peaks. As with all chromatographic techniques several combinations are necessary for reliable detection. However, when screening large numbers of samples this is seldom possible and so HPAEC has become a very convenient and acceptable monitoring tool.

Oligosaccharides and cane deterioration

Although serious processing problems can be attributed to the presence of some polysaccharides (dextran in particular), experience at the SMRI indicates that oligosaccharides play a dominant role in sucrose crystal habit modification (and thus also contribute to reduced growth rates). The introduction of HPAEC profiling opened the way for extensive monitoring and this technique was used to screen large numbers of juice samples in an attempt to show that post-harvest delays of whole stalk cane result in accumulation of oligosaccharides causing crystal elongation. In this section only one aspect of cane deterioration is discussed, viz. the accumulation of specific oligosaccharides during the delays between harvesting and crushing windrowed whole stalk cane (either trashed or burnt). Harvesting practices such as burning, topping or billeting as well as environmental conditions such as post-harvest temperature and rainfall will affect the rate of deterioration. However, none of these factors was examined and the following results are an attempt to extract general trends from a number of trials carried out under realistic industrial conditions. Limited trials indicated that variety might have an effect on the extent of oligosaccharide formation. This aspect has not been further investigated (3).

Oligosaccharides were measured on direct analysis of cane (DAC) extracts (1). The concentration of some oligosaccharides found in juice was found to be dependent on the method of extraction. This is discussed in the next section. However, DAC extracts generally gave consistent results. The cane was sampled several times a week for periods up to five weeks. Trials were conducted over extended periods in order to enhance the effects measured. In discussing the formation of oligosaccharides, comments have been limited to those oligosaccharides readily detected by HPAEC since this technique lends itself to monitoring large numbers of samples with minimal sample preparation. Furthermore, the study has been limited to those oligosaccharides observed in the sugar crystal (discussed in the next section) as these will play some part in habit modification.

The emphasis during most trials was on comparisons between the behaviour of burnt and unburnt cane. The results for the DAC extracts from these trials have been published (13). Data from typical trials are presented in Table 6. There were no obvious differences in the types of oligosaccharides found in freshly harvested or stale cane or in other factory products.

The three kestoses (1-, 6- and neo-kestose) were the main deterioration products and further discussion is limited to these oligosaccharides. Although other PAD responsive compounds formed, they were not detected in the sugar. High 1-kestose levels would obscure the presence of theanderose when using HPAEC monitoring and this oligosaccharide was later measured in evaporator syrup using GC (see next section). The predominant oligosaccharide in both burnt and trashed cane was 1-kestose. All three kestoses formed more rapidly in deteriorating burnt cane than in green cane subjected to similar post-harvest conditions. After excessive delay there were indications of nystose formation. Previous trials where thin layer chromatography or GC was used to monitor oligosaccharide formation gave qualitatively similar indications. Typical trends with delay time are shown in Figure 4. Although increased 1-kestose levels were typical of deteriorating cane, the concentration in fresh cane varied widely even for juices of similar purity and so its presence is not a useful indicator of cane deterioration. However, cane delays will inevitably lead to increased oligosaccharide loads to the factory.

The increases in kestose levels as cane purity decreased are presented in Table 7. The accumulation of 6-kestose and neo-kestose relative to the sucrose lost was considerably less when trashed whole stalk cane deteriorated. Burning or trashing had little effect on the relative extent of the major product, 1-kestose, but 6-kestose or neo-kestose formed more rapidly in burnt cane.

The accumulation of oligosaccharides during post-harvest delays is only one manifestation of enzymic, bacterial or microbial activity. It is undoubtedly accompanied by the formation of other products such as acids and polysaccharides (e.g. dextran or sarkaran). Conditions -- mechanical damage, cane maturity, temperature, humidity, rainfall etc. -- will dictate the major degradation pathways. Oligosaccharides are not the only contributor to processing problems. They are unlikely to have an effect on viscosity or mass transfer, but they will affect crystal shape and hence crystallisation rates.

Oligosaccharides and processing

Attempts have been made to examine what impact the presence of oligosaccharides may have on aspects of cane processing and sugar quality. Cane from several trials was processed in the SMRI pilot plant. Details have been published (7). The pilot plant can be used to produce A-sugar (of consistent crystal size) and A-molasses from cane stalks so that comparative investigations can be carried out. Findings from these trials as well as from factory or refinery experience have been used to postulate the interaction between oligosaccharides and processing. Typical results are summarised in Table 8. The following points can be made.

Cane preparation and extraction. The pilot plant includes a small three-roller mill, a shredder and a press for the extraction stage. About 50 kg of cane are fed through the mill. The juice is collected and the crushed stalks are soaked in 20 kg imbibition water (containing bactericide) and again fed through the mill. A further 15 kg of imbibition water is added to the bagasse, which is milled again. The final bagasse is fed through the Jeffco cutter grinder to give a pulp. This pulp is then pressed in a Pinette Emidecau hydraulic press (one minute at 200 kN/230 bar). The resultant press juice is added to the mill juice (mixed juice). Typical extractions ranged from 92 to 94 which is, not unexpectedly, lower than that achieved industrially.

In earlier deterioration trials cane samples were shredded in a Jeffco shredder and the juice was extracted in a cold digester (DAC extract), or with a Pinette Emidecau hydraulic press (press juice). These juices were analysed, but were not used for any processing trials. A significant aspect of these trials was that the levels of 1-kestose in press juice were frequently considerably higher than those in DAC extracts. The effect was particularly noticeable when badly deteriorated cane was used. A preliminary run with the pilot plant mill showed similar effects for mixed juice (Figure 5). This was attributed to microbial or enzymic activity as a result of the ideal incubation conditions present during preparation and extraction of infected cane. During subsequent trials precautions were taken to minimise this effect by dosing the imbibition water with a bactericide (Busan 881). It can be seen from Figure 5, where 1-kestose concentrations in DAC extracts and mixed juice are similar, that such treatment was generally successful in preventing further oligosaccharide formation during extraction.

Cane delays are the major source of oligosaccharides, but extraction conditions also influence the type and level of oligosaccharides introduced into process. Mill sanitation would be particularly important when crushing deteriorated cane, and biocide treatment in the caneyard or at the shredder might be beneficial. Parallels exist between these results and milling or diffusion. It is possible that the higher temperatures maintained in a diffuser probably inhibit enzymic activity and so limit oligosaccharide formation during extraction. By contrast, conditions in a milling tandem are probably conducive to microbial and enzymic activity and thus enhance the accumulation of oligosaccharides in mixed juice from deteriorated cane.

Clarification and evaporation. Clarification was by lime defecation in a 150 litre tank. The juice was heated to boiling, milk of lime was added to adjust the pH to 7.1 to 7.2 and the juice was boiled for a further two minutes. After adding flocculant (5 - 10 ppm), the mud was allowed to settle for 30 minutes. Clear juice was fed via a holding tank directly to the evaporator. Evaporation was done in a two effect evaporator with a

capacity of 12 litres per hour. Two passes were necessary to produce syrup of 60 - 65° Brix. Syrups produced from deteriorated cane showed increased levels of trisaccharides, some intermediate MWt material together with considerable increases in material with MWt > 7000. This can be seen in Figure 6 where size exclusion profiles (Fractogel HW 40) are shown. Since the high 1-kestose levels obscured the theandrose peak in juice or syrup samples from deteriorated cane when using HPAEC, GC was used to measure theandrose in evaporator syrup. Levels in syrup were relatively unchanged as deterioration increased (Table 8). Similar behaviour has been reported previously (2).

Kestoses were destroyed during clarification, with 1-kestose being most affected (Figure 7). This sensitivity to hot alkaline degradation would probably also apply to sucrose. Eggleston (5) has recently reported similar alkaline degradation of 1-kestose across clarification. There were further small decreases during evaporation. The destruction of 1-kestose was also observed during laboratory trials in which factory molasses was heated at 70°C for extended periods (12). This was accompanied by an increase in theandrose (Table 9). Similar effects have been noticed during refining (see next section). In general, however, processing (other than extraction as discussed above) had little effect on oligosaccharide levels. In broad terms, increases in input levels were reflected throughout process. Any destruction was relatively minor when compared to the large increases associated with the initial cane delay.

Crystallisation and oligosaccharide transfer. The main impact of the presence of oligosaccharides will be experienced during crystallisation. Smythe (17) has shown that some oligosaccharides poison specific faces of the sucrose crystal causing habit modification which leads to reduced crystal growth rates. It has been demonstrated that crystal growth rates in South African refineries are adversely affected by oligosaccharides (9). Impurity transfer to the crystal will depend on many factors. One of these factors will be the concentration of the impurity in the feed liquor. With the controlled conditions maintained in the pilot plant, the relative importance of various impurities can be compared. The transfer of sugars from the feed to the crystal under pilot plant conditions is presented graphically in Figures 8 and 9. The slopes of these plots represent the amount of oligosaccharides in the crystal as a fraction of that in the mother liquor during boiling. The values are not absolute, but will change with conditions (Table 10). The use of an approximately linear relationship is adequate to indicate trends. The increased levels of oligosaccharides resulting from cane deterioration are reflected in higher oligosaccharide levels in evaporator syrup with concomitant higher concentrations in the crystal. Those oligosaccharides with the highest slopes show the greatest transfer.

Figure 8 illustrates that, although large amounts of monosaccharides are present in syrup from stale cane, their transfer to the crystal is clearly much less than that of the oligosaccharides. It can be seen from Figure 9 that 1-kestose (the major deterioration product) is adsorbed less strongly than theandrose, 6- or neo-kestose (about 25 to 30% as much). These last two oligosaccharides are formed rapidly in deteriorating burnt cane. Reduced crystal growth rates will be encountered when processing such cane.

Theandrose has been found to be the predominant oligosaccharide in cane sugar (14). Only sugars prepared from severely deteriorated cane showed kestose concentrations higher than those of theandrose (Table 11). The strong tendency of theandrose to transfer to the crystal is shown in Table 12 where it can be seen that about 90% of the theandrose present in VHP is associated with the affinated crystal. By comparison about

20% of the neo-kestose and 40% of the 1-kestose is present in the molasses film. It is this chemisorption effect shown by some oligosaccharides during crystallisation that inhibits growth on specific faces and causes crystal habit modification. Mantovani et al. (8) have indicated that the contributions of glucose, fructose and dextran to sucrose crystal morphology are probably due to a competitive mechanism and are less important than other major elongating effects observed in industry. They probably exert some influence on growth, but are not necessarily transferred.

Although the main source of oligosaccharides in mixed juice comes about as a result of cane delays and deterioration, some seasonal physiological contribution is also to be expected. However, whatever the origin, the effects can be expected to be detrimental. Pilot plant experiments have indicated that the oligosaccharide concentration in processing streams

- can be enhanced by the extraction technique
- may be influenced during clarification
- will affect crystal growth rates.

Refining. The quality of refinery melt will depend on the quality of the raw input. Certain oligosaccharides tend to transfer preferentially to the crystal so that the VHP sugar entering the refinery is enriched with compounds that will prevent efficient crystallisation. This enrichment is depicted in Figure 10 where visual inspection shows that most of the oligosaccharides present in factory massecuite are excluded from the crystal during crystallisation as is to be expected, but relatively more theandrose than 1-kestose is transferred to the raw crystal. Analysis of massecuites and jets from a carbonation refinery (15) showed an apparent increase in the ratio of 1-kestose to theandrose (Table 13). This is in contrast to the decrease in this ratio observed when factory C-molasses was heated (previous section). The ratios of both 1-kestose to theandrose and 6-kestose to theandrose show an increase across refining, whereas the ratio of neo-kestose to theandrose is unchanged. This indicates the selective removal of theandrose and neo-kestose across refining. Unfortunately no mass balances were carried out, so that it is uncertain whether this is due to crystal transfer or to degradation. The changes in these ratios are difficult to explain. Ion exclusion chromatography (strong cation resin in the sodium form) was used to separate refinery jets into two fractions - an excluded fraction (A) and a retained fraction (B) (18). The excluded fraction will contain polymers, colorants and ash, whereas the retained fraction includes oligosaccharides, sucrose and some monosaccharides. It can be seen from Figure 11 that Refinery 2 jet contains considerably more theandrose than that of Refinery 1. Furthermore, the 'oligosaccharide' peaks in Refinery 1 jet are relatively broad. Figures 12 and 13 illustrate that this peak broadening is caused by compounds 1a and 2a which elute closely to peaks 1 (1-kestose) and 2 (theandrose) respectively. Since they are removed by ion exclusion chromatography they are probably ionic, e.g. saccharinic acid breakdown products. Refinery 2 jet shows no such compounds.

Theandrose has been found to transfer strongly to the cane sugar crystal and can be detected in the purest cane sugar (14). Beet and cane white sugar HPAEC profiles are shown in Figure 14. The raffinose peak is typical of beet sugars, whereas the theandrose peak has been found to be characteristic of cane sugars.

The evidence presented here illustrates that the effects of oligosaccharide accumulation during cane deterioration (and, to a lesser extent variety and maturity differences) will carry through to the sugar and ultimately to the refinery where their influence on crystallisation rates will be particularly marked. Impurities associated with cane processing cannot be avoided, but the effects of oligosaccharides can be reduced by minimising cane delays and maintaining good mill sanitation.

CONCLUSIONS

Oligosaccharides have been shown to be a major cause of crystal elongation, particularly in refining. HPAEC has been used to measure these sugars in a variety of products and some analytical precautions have been highlighted.

Although there are undoubtedly some seasonal and varietal differences in oligosaccharide concentrations in cane juice, the main source is as a result of deterioration during cane delays. The kestoses were the main oligosaccharides formed. The rate of formation of the kestoses was faster for burnt than green cane. Similar amounts of 1-kestose were formed per unit purity drop with either treatment. However, two to three times as much 6- or neo-kestose was formed per unit purity lost when burnt, compared with green, cane deteriorated. Theandrose did not appear to increase as cane deteriorated. The method of juice extraction and mill sanitation played some part in increasing juice oligosaccharide levels, particularly when stale cane was processed. There were indications that small decreases in oligosaccharides occurred during clarification. In general, the concentrations throughout process were related to the amounts included with the cane.

Increased oligosaccharide loads to the factory adversely affect crystallisation rates by selective adsorption on different crystal faces. Some oligosaccharides (e.g. 6-kestose, neo-kestose and theandrose) were transferred more readily than others such as 1-kestose, leading to crystal elongation and retarded crystal growth rates. The extent of this transfer was related to the concentration in the feed liquor. By contrast, despite high fructose and glucose levels in syrup from deteriorated cane, very little invert was transferred to the crystal.

Oligosaccharides in the crystal are subsequently transported to the refinery where they are rapidly concentrated, leading to exhaustion difficulties. The variability in oligosaccharide concentrations in sugars is reflected in refinery melt. Oligosaccharides continue to be concentrated throughout the refining process, exacerbating slow crystallisation rates. Theandrose is so strongly adsorbed that it was evident in all cane sugar crystals examined. There is evidence that acidic breakdown products are formed during refining. These are PAD responsive and can interfere in HPAEC analysis of oligosaccharides.

Hence it can be seen that increased juice oligosaccharide concentrations, which occur mainly as a result of cane delays, will influence crystallisation rates right through factory processing and ultimately into refining.

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Table 1. Effect of molasses fractions on crystal shape and growth rate.

Fraction	Crystal shape (c/b)	Growth rate ($\times 10^5$) ($\text{kg.m}^{-2}.\text{s}^{-1}$)
Sucrose	0.54	5.7
Molasses (unfractionated)	1.33	3.1
H M Wt (polysaccharides)	0.51	6.5
L M Wt	1.25	4.2
Mono- + disaccharides	0.59	5.1
Oligosaccharides	1.11	2.2
H M Wt + L Mwt (i.e. recombination)	1.33	

Table 2. Influence of invertase hydrolysis on the crystal habit modifying properties of the oligosaccharide fraction.

Preparation	Crystal shape (c/b)
Sucrose	0.54
+ Oligosaccharide fraction	1.11
+ Oligosaccharide fraction + buffer	0.91
+ Oligosaccharide fraction after invertase hydrolysis	0.51

Table 3. Distribution of dextran (AOAC) in raw sugar samples.

Sugar	1	2	3
H Mwt (50% ethanol) (ppm)	55	25	90
Total (80% ethanol) (ppm)	275	120	255

Table 4. Observations on dextranase treatment.

Trial type		Refinery (HV)	Lab (NB)	Lab (SZ)
Product		white boiling (1987)	C - M/C (1991)	A - M/C (1996)
Polysaccharides	before	-	31,000 ppm	6,300 ppm
	after	-	20,000 ppm (as total polysac)	4,500 ppm (as AOAC dextran)
Viscosity	before	-	14 Pa.s	410 cP
	after	-	8 Pa.s	310 cP
Crystal shape (c/b)	before	0.72	2.0	-
	after	0.72	1.8	-
Comments		Refinery reported process improvements	Confirmed dextran	Confirmed dextran

Table 5. Crystal shape in fractionated factory massecuite .

	c/b ratio	
	Aug	Oct *
C - massecuite (factory @ ca. 55 purity)	1.7	2.5
C - massecuite (laboratory @ ca. 78 purity)	1.0	1.2
Polysaccharide fraction	0.5	0.6
Oligosaccharide fraction	0.7	1.1
Oligosaccharides (% NS)	1.6	4.1

* severe processing problems were being experienced

Table 6. Accumulation of kestoses in DAC extracts during delays of burnt or trashed cane.

Trial	3							
Treatment	Burnt				Trashed			
Day	Pty	Oligosaccharide (ppm on Bx)			Pty	Oligosaccharide (ppm on Bx)		
		1-K	6-K	n-K		1-K	6-K	n-K
0	91.1	3920	295	520	89.1	3185	565	745
2	88.2	3180	420	460	89.4	2930	395	720
5	83.6	4295	690	665	84.4	4345	385	850
7	85.8	5855	790	1080	82.3	5495	655	925
9	83.5	6670	800	1690	75.7	7025	865	1285
12	83.1	6735	775	1770	77.4	5570	555	1045
14	80.5	7280	1845	3185	76.3	5770	760	1575
16	78.7	7790	1365	2575	70.0	7450	765	1755
19	69.4	9995	2310	4680	73.3	7510	1045	1700
21	67.7	8460	3475	6710	69.0	8415	1295	2505
23	59.3	9415	3460	7080	68.7	9515	1095	2025
26	49.6	10590	3685	6070	63.3	9050	1775	2745
28	55.1	6720	1790	3185	63.4	4900	735	1335

Trial	4							
Treatment	Burnt				Trashed			
Day	Pty	Oligosaccharide (ppm on Bx)			Pty	Oligosaccharide (ppm on Bx)		
		1-K	6-K	n-K		1-K	6-K	n-K
0	89.2	2750	355	350	88.9	3930	510	630
2	89.5	2660	400	330	84.6	5535	1245	745
4	86.5	3700	590	475	83.8	5720	1190	840
8	84.2	3725	495	535	85.6	4520	460	775
10	85.7	4675	570	940	85.0	4055	355	720
14	76.2	6845	1025	1455	81.4	6875	885	1175
16	77.1	6505	1175	1655	84.3	6395	770	1180
18	80.7	5450	895	1780	76.5	8090	1335	1215
21	69.9	10485	3485	5020	67.9	9580	1875	2000
25	64.7	8325	3560	6900	71.0	7435	1325	2030
28	64.5	7740	2065	3325	68.8	8710	1430	2665

Table 6. (continued)

Trial	5							
Treatment	Burnt				Trashed			
Day	Pty	Oligosaccharide (ppm on Bx)			Pty	Oligosaccharide (ppm on Bx)		
		1-K	6-K	n-K		1-K	6-K	n-K
0	90.7	835	260	200	90.5	895	285	185
1	91.1	1005	220	165	90.8	750	145	170
2	91.7	945	160	175	90.1	1235	415	230
3	91.1	1230	175	245	90.6	1000	180	240
7	90.1	4485	390	725	87.8	2505	375	500
10	85.3	9065	540	1055	82.2	3045	380	650
14	80.6	7685	620	1660	76.8	4365	470	855
17	74.1	7025	645	1600	74.3	4740	465	935
21	68.7	13665	1645	3925	71.8	8395	700	1390

Trial	6							
Treatment	Burnt				Trashed			
Day	Pty	Oligosaccharide (ppm on Bx)			Pty	Oligosaccharide (ppm on Bx)		
		1-K	6-K	n-K		1-K	6-K	n-K
0	87.4	650	210	60	74.7	650	190	225
1	87.5	565	165	170	87.5	770	195	245
2	86.4	845	150	170	86.2	1160	230	415
3	87.5	670	110	215	86.7	1315	160	425
7	84.2	5780	1345	1000	81.9	3040	370	655
10	76.7	8035	3300	2260	74.7	5165	675	1085
14	70.1	9225	5365	3835	72.8	5935	715	1170
17	67.1	8020	5850	3930	69.8	7125	945	1375
21	64.1	6380	4185	3175	65.9	7325	1020	1596

Table 7. Kestose formation as a function of sucrose destruction (mg/g sucrose destroyed) as cane deteriorates.

	Treatment	
	Burnt	Trashed
1-kestose	26	26
6-kestose	12	3
neo-kestose	19	6

Table 8. Oligosaccharides in process products prepared from deteriorated cane.

1-kestose (ppm on Bx)					6-kestose (ppm on Bx)				
D	M	C	E	S	D	M	C	E	S
1280	1275	995	1200	250	390				155
620	1340	865	710	115	325	465	400	290	
2970	2375	1995	2075	160	1155	870	805	625	120
3415	2620	2345	2355		2410	1340	1125	1115	
3460	3190	2890	2860	165	985	765	865	850	80
5900	3525	2870	2800	175	1560	540	475	490	105
5730	4890	4620	5120	260	705	600	580	595	105
3420	5350	3000	2645	140	435	440	500	410	
7100	6465	5515	5260		4145	2830	2930	2730	
6370	6500	5780	5445	460	895	790	750	680	145
2080	7710	4000	4240	220	540	620	510	660	50
2300	9005	5110	4405	565	1310	2065	1790	1630	275
3265	9075	4785	4500	370	1045	1165	920	855	135
2400	9850	4970	5210	500	1390	1665	1285	1300	180

Table 8. (continued)

neo-kestose (ppm on Bx)					Theandrose (ppm on Bx)	
D	M	C	E	S	E	S
700	595	555	595	200	830	205
420	580	565	400	70	1060	240
2790	1800	1565	1525	270	960	225
2165	1635	1485	1465			
1880	1495	1340	1440	240	890	240
1520	1225	1065	1060	190	595	145
1495	1260	1155	1285	290	825	225
1895	1630	905	885	145	845	240
4665	4490	4610	3860			
1590	1735	1580	1585	485	430	150
1125	2060	1185	1415	300	1000	260
2695	4000	3215	3015	830	1165	275
2225	2925	1955	1955	520	1055	270
2850	3600	2500	2535	680	1185	280

Table 9. Changes in concentration of 'oligosaccharide' peaks when C-molasses is heated at 70°C.

(Retention relative to panose)	Oligosaccharide (% sample)		
	1-kestose (0.46)	theandrose (0.47)	unknown (2.23)
Sample 1: before	0.80	0.45	0.75
after	0.55	0.70	0
Sample 2: before	0.60	0.50	0.23
after	0.55	0.70	0
Sample 3: before	0.70	0.40	0.52
after	0.55	0.70	0

Table 10. Relative transfer of oligosaccharides to the sucrose crystal.

Sugar	Slope	n	r
Glucose	0.024	16	0.91
Fructose	0.028	16	0.92
1-Kestose	0.300	24	0.66
6-Kestose	0.870	24	0.77
neo-Kestose	1.300	24	0.87
Theandrose	1.530	15	

Table 11. Oligosaccharides in cane sugar (pilot plant) prepared from deteriorated cane.

Cane delay (days)	Oligosaccharide (ppm)			
	Theandrose	1-Kestose	6-Kestose	neo-Kestose
0	240	115		70
7	240	140		145
14	260	220	50	300
21	270	370	135	520
28	280	500	180	680
35	275	565	275	830

Table 12. Distribution of oligosaccharides in cane raw sugar.

	Crystal	Molasses film
Theandrose (%)	92	8
neo-kestose (%)	78	22
1-kestose (%)	62	38

Table 13. Changes in oligosaccharide ratios during refining.

Refinery massecuite	Oligosaccharide ratio			
	1-K/Th	6-K/Th	n-K/Th	1-K/6-K
1	0.8	0.5	0.8	1.6
2	0.6	0.5	0.7	1.1
3	0.9	0.6	0.8	1.6
4	0.9	0.5	0.6	1.6
5	1.3	0.9	0.7	1.5
6	1.4	1.0	0.7	1.4
7	1.6	1.2	0.7	1.4

1-K = 1-kestose, 6-K = 6-kestose, n-K = neo-kestose, Th = theandrose.

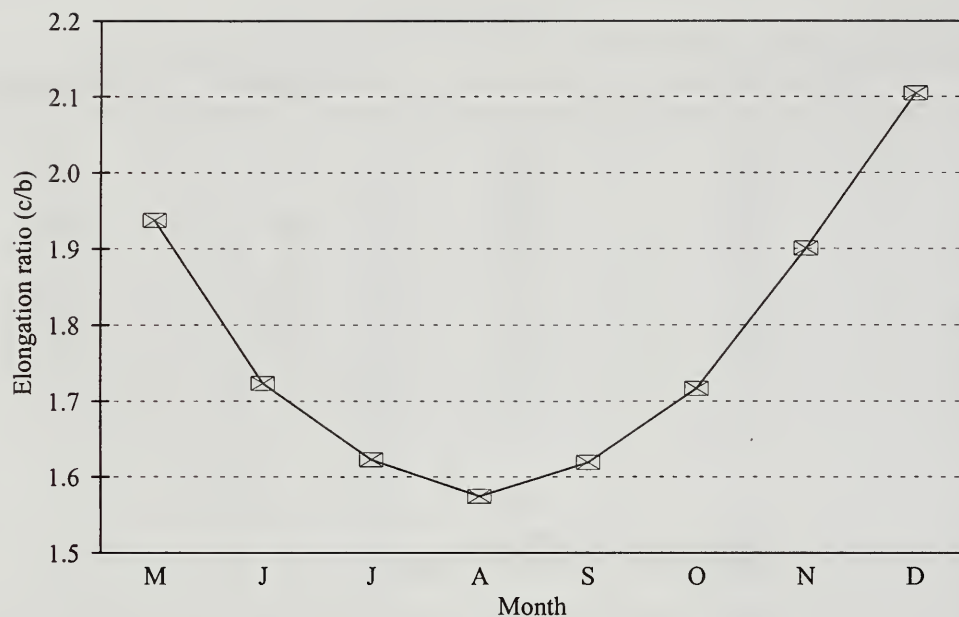


Figure 1. Seasonal trend in C-sugar crystal shape. Industrial average for the period 1984 to 1997.

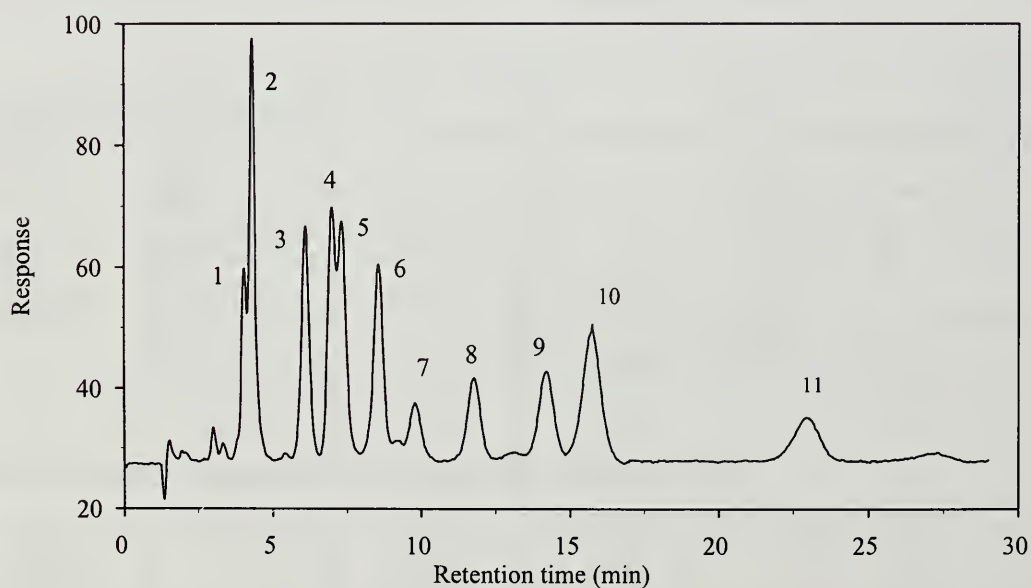


Figure 2. HPAE chromatogram of a mixture of reference oligosaccharides (Solvent: 100 mM NaOH/15 mM NaOAc). 1 = isomaltose; 2 = sucrose; 3 = raffinose + isomaltotriose; 4 = 1-kestose; 5 = theanderose; 6 = maltose; 7 = isomaltotetraose; 8 = 6-kestose; 9 = neo-kestose; 10 = isomaltopentaose + panose; 11 = maltotriose.

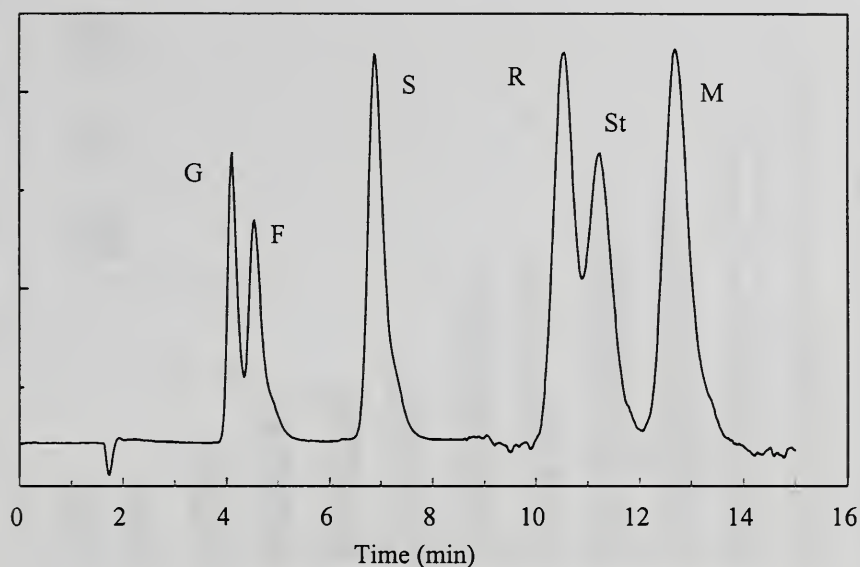


Figure 3. HPAE chromatogram using 150 mM NaOH/10 mM NaOAc showing separation of (1) raffinose & stachyose (not separated with NaOH < 125 mM) (2) stachyose & maltose (not separated with NaOH > 160 mM). G = glucose, F = fructose, S = sucrose, R = raffinose, St = stachyose, M = maltose.

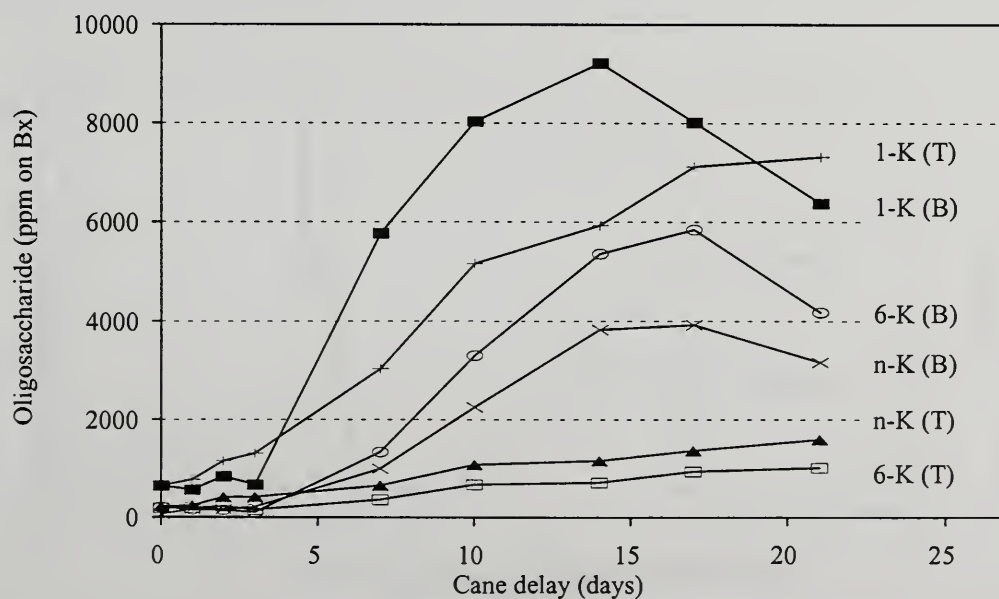


Figure 4. Kestoses in DAC extract during deterioration of burnt or trashed windrowed cane (1-K = 1-kestose; 6-K = 6-kestose; n-K = neo-kestose; B = burnt; T = trashed).

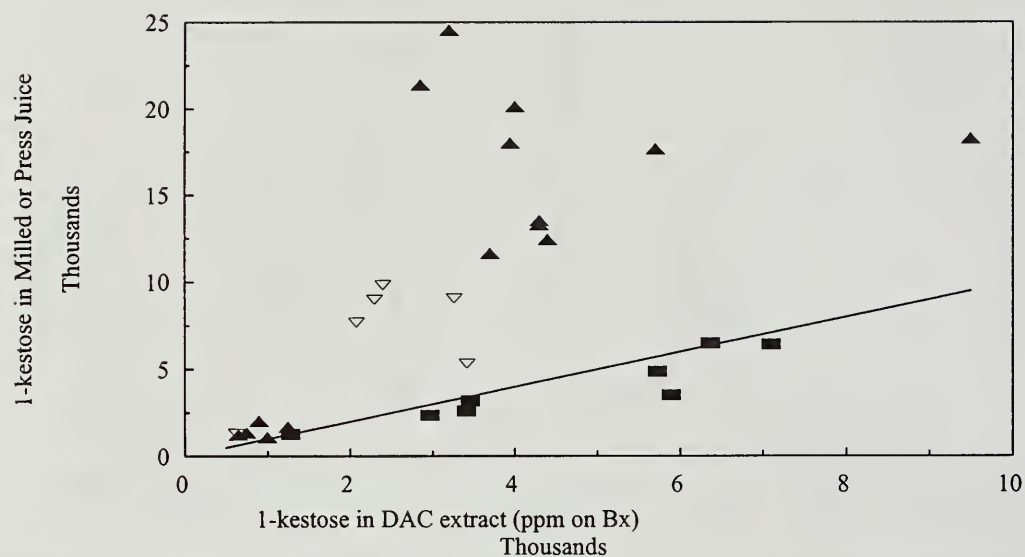


Figure 5. Comparison of 1-kestose concentrations in DAC extracts, mixed and press juice from deteriorated cane. (a) press juice (no bactericide) ▲; (b) mixed juice (no bactericide) ∇; (c) mixed juice (with bactericide) ■.

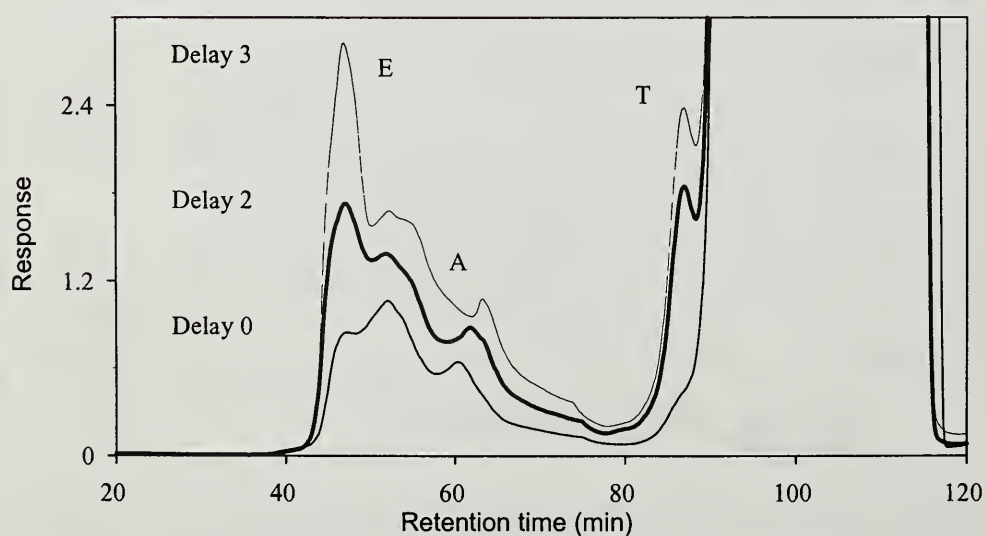


Figure 6. Size exclusion profile of syrups prepared from deteriorated cane. T = trisaccharides; A = ash; E = excluded peak (includes polysaccharides)

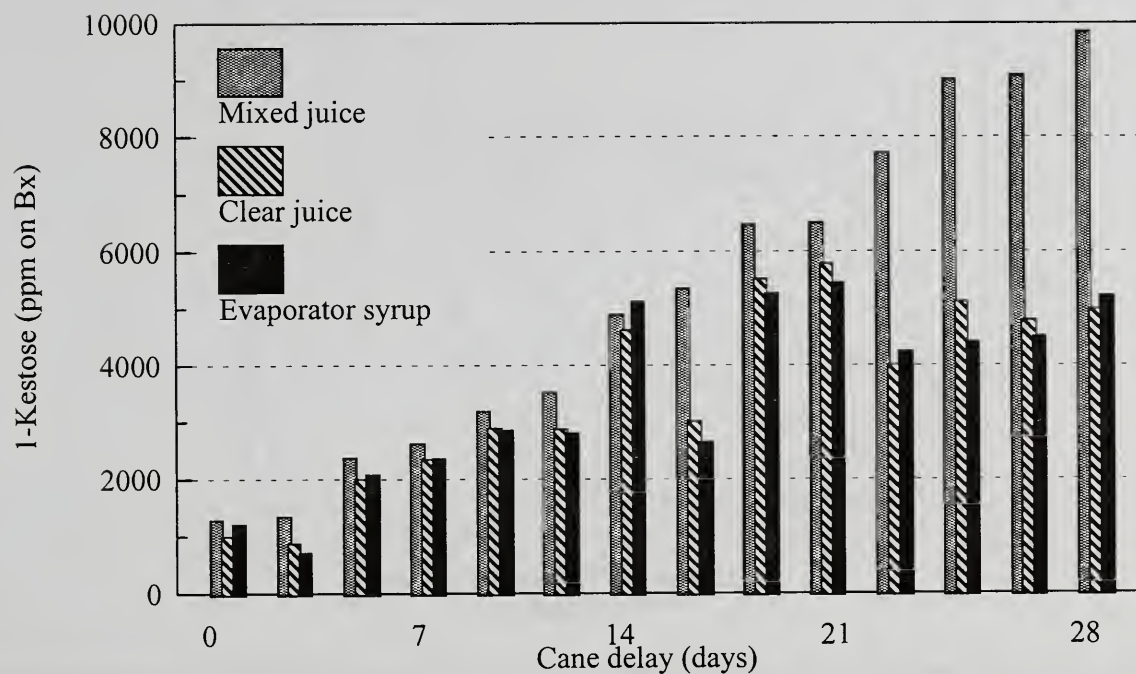


Figure 7. 1-kestose concentration in mixed juice, clear juice and evaporator syrup when processing deteriorated cane.

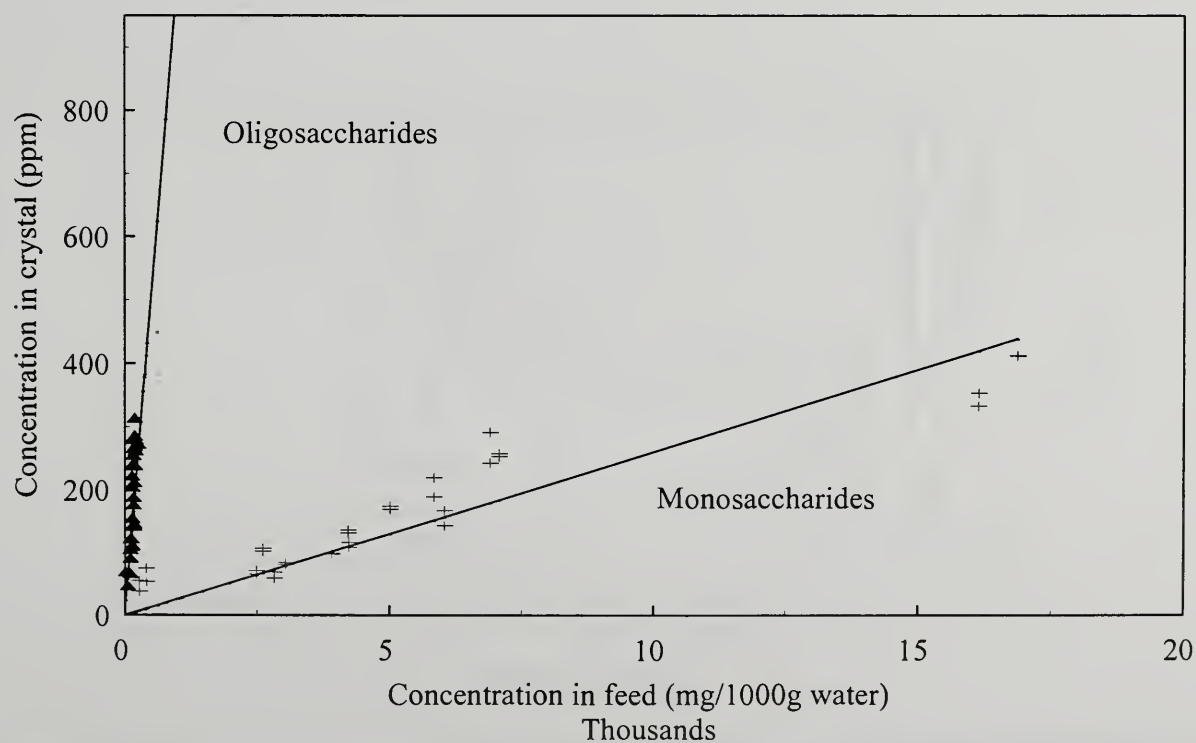


Figure 8. Transfer of monosaccharides (+) and oligosaccharides (▲) from mother liquor to the crystal during crystallization.

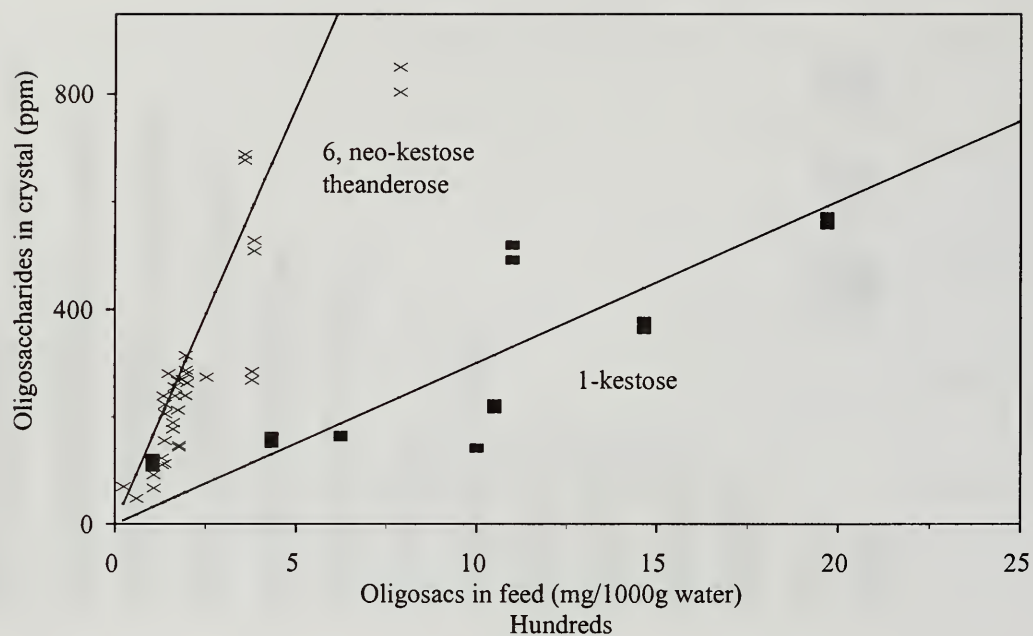


Figure 9. Comparison of transfers of 1-kestose (■) and other oligosaccharides (x) from mother liquor to the crystal during crystallization.

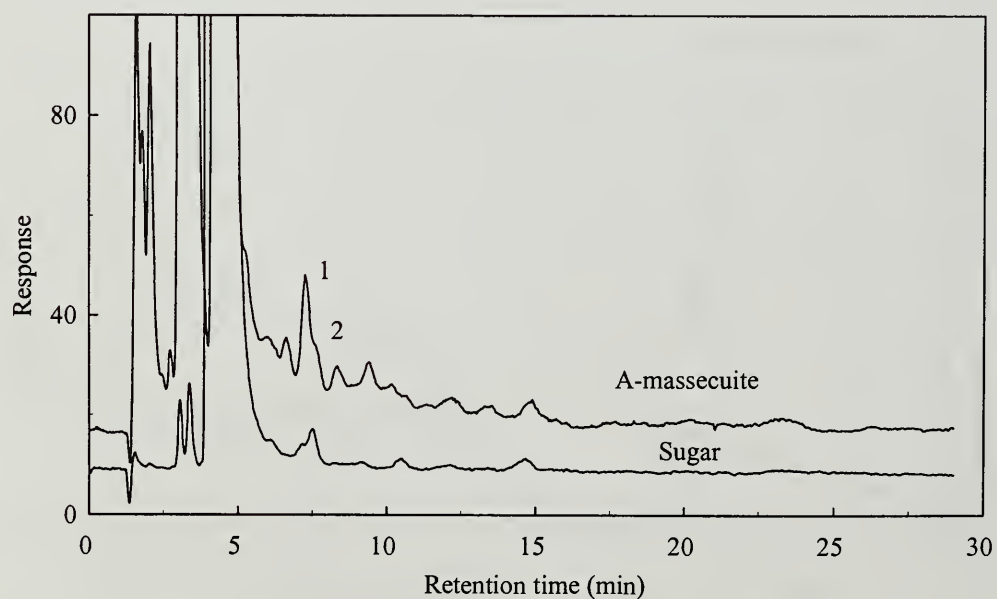


Figure 10. HPAEC profiles of oligosaccharides in A massecuite and A sugar. 1 = 1-kestose; 2 = theandrose.

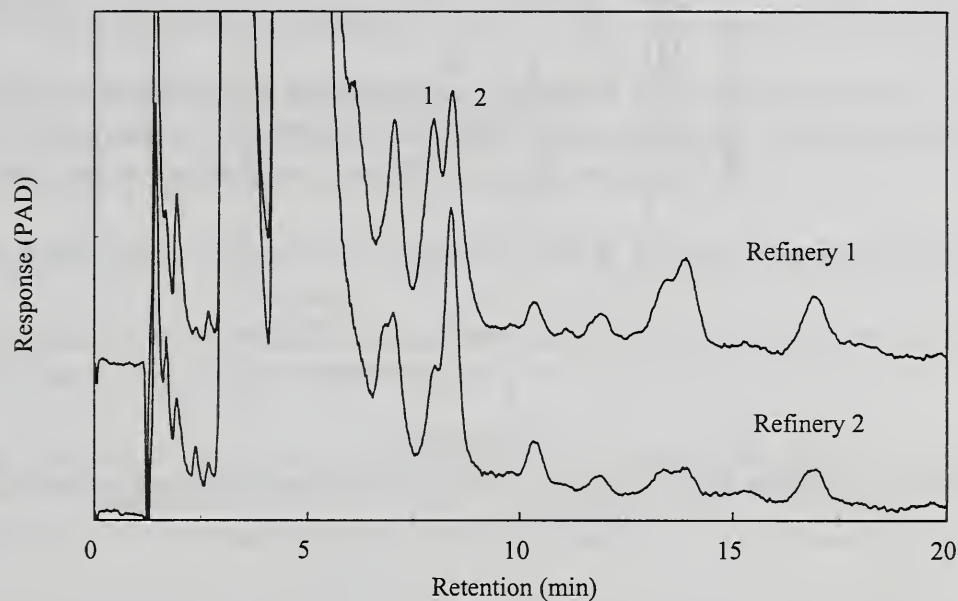


Figure 11. HPAEC profiles of Jet 4 from two refineries showing different ratios of 1-kestose and theandrose. Peak 1 = '1-kestose', Peak 2 = 'theandrose'.

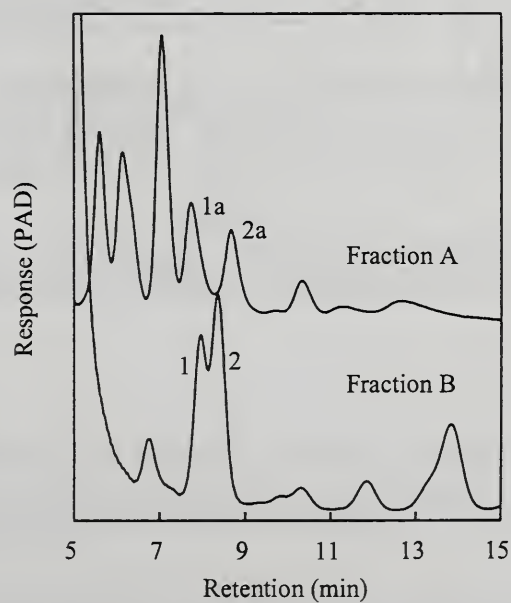


Figure 12. Ion exclusion fractions from Refinery 1 Jet 4 showing large amounts of excluded components (peaks 1a and 2a).

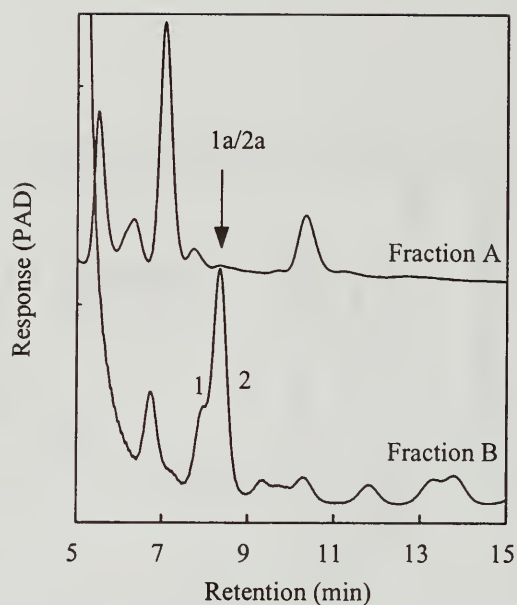


Figure 13. Ion exclusion fractions from Refinery 2 Jet 4 showing negligible amounts of peaks 1a and 2a.

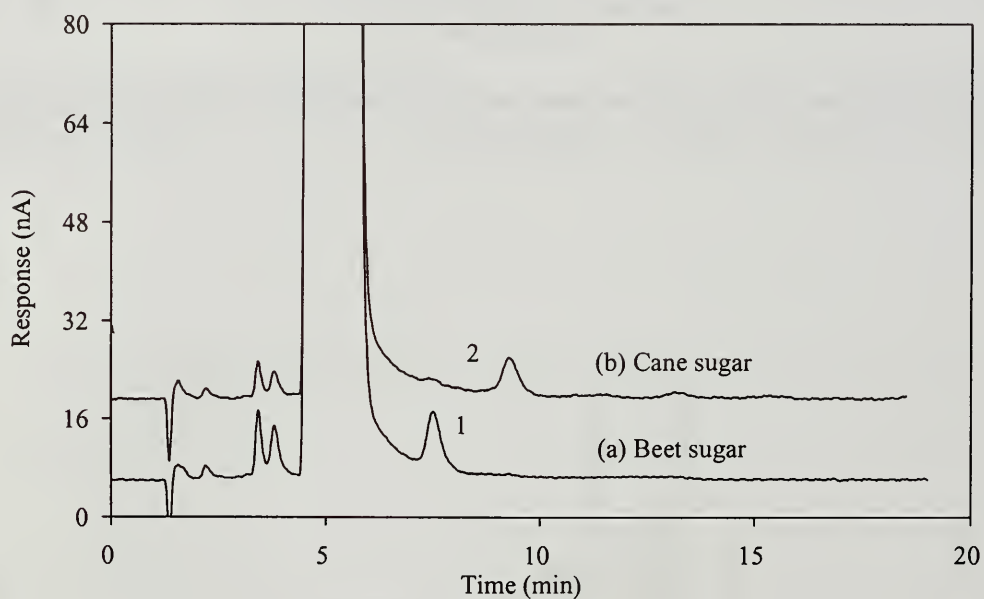


Figure 14. HPAEC profiles of cane and beet sugars. 1 = raffinose (370 ppm); 2 = theanderose (345 ppm).

DISCUSSION

Question: What effects do these oligosaccharides have on the pol of raw sugar using lead acetate and using other clarification aids?

Morel du Boil: The kestoses, as far as I can recollect, would affect pol by something like half a unit per unit of kestose - actually 0.4 units. We have no information on the influence of different clarifying reagents.

Question: Kestose is important in the acid degradation of sucrose. Sucrose breaks down into glucose and fructose carboxonium ions, and then the fructose carboxonium ion reacts with another sucrose molecule to form kestose. You said that the bacteria in the process stream are increasing kestoses. Is that because it is inducing acid degradation of sucrose, affecting the pH, or have you come up with another mechanism?

Morel du Boil: No, we presume this is an enzyme reaction. It is well known that invertase, and some amylases, catalyse the formation of kestoses in solution. These enzymes can be present in the cane and are also formed by yeasts and/or bacteria as a result of cane deterioration.

Question: Is there a difference in kestoses in varieties - that is, is there a varietal effect?

Morel du Boil: Not that we have noticed, but we have not done a lot of work on varieties. I would think that maturity would have a bigger effect than variety.

Question: I am not familiar with the kestose evaluation or the degradation of the cane, but I just wanted to get an idea. In one of your slides, you show a value of the 6-kestose as 12 and the neo-kestose as 19. Can you give me some idea of a benchmark so I can evaluate whether 12 and 19 are high or is it normal?

Morel du Boil: Those numbers are related to the amount of sucrose that is destroyed. You are making 12 or 19 mg of kestose per g of sucrose destroyed. You can see from the green cane that the figures were down to 2 or 3 mg.

Question: So the comparison would be between 2 or 3 and 12 and 19?

Morel du Boil: Yes, in that range.

Question: What would be a baseline level of theanderose compared to the kestoses?

Morel du Boil: I do not recall exact figures, but we do detect it all the way through the process. It is generally the highest oligosaccharide present. As soon as the cane starts to go off, the kestoses start to become the predominant oligosaccharides.

Question: The reason I asked is that the affinity of theanderose in the crystallization process for the face of the crystal growth must be competitive with the other kestoses.

Morel du Boil: Yes.

Question: You gave the profiles of two different refinery operations and you said there was more degradation in one refinery than the other. Were they processing the same raws or were the raws from different sources?

Morel du Boil: The raws were from different sources, but essentially the melt profile is very similar.

EVALUATION OF CANE TRASH

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ABSTRACT

Extraneous matter, consisting of tops, leaves and field soil, incorporated with commercial cane during harvest reduces the efficiency and sugar recovery at the factory. In order to control extraneous matter, a reliable low cost evaluation method is required. The core sampler is frequently used in mills to determine cane quality and was used as the basis for developing a simple method to determine extraneous matter in cane samples. Theoretical considerations indicated that the relation between sucrose content of a commercial cane sample and a cleaned sub sample should provide a reasonable estimate. Laboratory and field trials confirmed the potential of this methodology.

INTRODUCTION

Extraneous matter or field trash is defined as leaves, tops, dead stalks, roots, soils etc. delivered to the factory together with cane (3). Legendre (1,2) reported increased levels of field trash as a result of mechanical harvesting in Louisiana since 1943. Similar results have been obtained in other countries like in Australia (1).

Studies in Louisiana showed that with 8.5% of trash, the sucrose extraction was not affected, but the juice quality was reduced (1). Legendre and Irvine (2) reported that the fiber in clean cane increased by 56% when the trash was increased from 0% to 30%, and juice extraction and sugar recovery were reduced. Statistical and regression analyses showed that for each 10% of trash in clean cane there is a sugar loss of 15 kg per ton of cane (4). Similar studies of the effects of tops and trash on extraction juice quality and clarification by R.P. Scott and co-workers in Hulett's Sugar Limited (Mount Edgecombe) revealed that trash percentages between 2 - 5% for tops and 3 - 10% for any extraneous matter may cause seriously reduced juice extraction and negatively affect mixed and clear juice qualities (6).

The environmental conditions of the Cauca Valley, Colombia, favor deterioration and high levels of trash in cane, which may reduce juice quality and sugar recovery. The annual precipitation in the Cauca Valley is around 1000-2500mm with an average temperature of 24°C. In the Cauca Valley the rainy season (April - May) brings many problems in harvesting, including high levels of trash in the cane and sugar recovery decreases in all mills during the wet season in the Cauca Valley. Some years ago, the mills in Colombia established an evaluation system for cane quality, including trash in cane, based on cleaning the cane and weighing the trash of commercial cane samples, taken from wagons or trucks; this task is

time consuming and costly and a better method for estimating the trash in cane delivered to the factory is required.

MATERIAL AND METHODS

Theoretical approach to trash evaluation in sugarcane

The core sampler method for determining the quality of commercial cane determines the sucrose content of a cane sample. We reasoned that if trash is essentially material that does not contain sucrose, we could use the ratio of sucrose in clean cane and dirty cane to estimate the trash level of commercial cane. The procedure would be based on the standard core sample from a cane wagon and the sucrose content of a sample of cleaned cane. The mathematical basis of the model is outlined below. The following data were required for trash calculation :

- Weight of juice extracted from clean cane (J_o).
- Weight of juice extracted from dirty cane (J_c).
- Juice extracted (%) from clean cane (E_o).
- Juice extracted (%) from dirty cane (E_c).
- Fiber (%) in clean cane (F_o %).
- Fiber (%) in dirty cane (F_c %).
- Sucrose (%) in clean cane (S_o %).
- Sucrose (%) in dirty cane (S_c %).

In a sample of commercial cane, the total (S_c) is equal to the sum of the sucrose content from clean cane (S_o) and sucrose from the trash (S_t), such as tops and suckers, according to the following equation:

$$S_c = S_o + S_t \quad (1)$$

Then

$$W_c S_c (\%) = S_o (\%) \times W_o + S_t (\%) \times W_t \quad (2)$$

and

$$W_c = W_o + W_t \quad (3)$$

where W_o is the weight of clean cane and W_t the weight of trash and W_c the weight of commercial cane. By rearrangement and combination of the two equations:

$$\frac{W_t}{W_c} = \frac{S_o(\%) - S_c(\%)}{S_c(\%) - S_t(\%)} \quad (4)$$

Therefore, the percentage of trash in cane can be expressed in the following equations:

$$\text{Trash \% cane} = \left(\frac{S_o\% - S_c\%}{S_o\% - S_t\%} \right) 10^2 = \left(\frac{1 - \frac{S_c}{S_o}}{1 - \frac{S_t}{S_o}} \right) 10^2 \quad (5)$$

If the contribution of sucrose from the trash ($S_t\%$) is not significant or equals zero, the equation is reduced to:

$$\text{Trash \% cane} = \left(1 - \frac{S_c\%}{S_o\%} \right) 10^2 \quad (6)$$

This method is particularly attractive since the trash does not contribute to the sucrose content in significant proportions and therefore the equation would predict the level of trash by determining the sucrose in samples of dirty cane and clean cane from the same plot after harvesting.

Experimental approach for trash evaluation

The present study was divided into a laboratory scale trial and a commercial scale trial.

The laboratory trials were carried out using 13-14 month old samples of MZC 74-275, CCSP 89-1997 and CC 91-1999. Fresh green cane was harvested manually and taken to the laboratory where it was separated into clean cane stalks, tops, dry leaves, and soil. The tops, dry leaves and soil are considered to be trash.

The individual trash components were shredded separately in a Jeffco cutter grinder and mixed with a ratio of 3:1 of tops to other trash. The trash was then added to the clean cane to give a trash weight to clean cane of 0, 1, 3, 5, 10, 15 and 20 percent. Three replications of 1000 g of the shredded material was used for each treatment. The following parameters were determined using the standard methodology of the core sampler (1,4): juice weight, fiber % cane, brix and sucrose of the primary juice (obtained by pressing in a hydraulic press at 3000 p.s.i.).

For the commercial trial, 109 commercial cane samples from hand cut burnt cane delivered to the Central Castilla sugar mill were analyzed with the core/press method developed in Louisiana (4). Each cane sample delivered to the mill was sampled from wagons by the core-sampler. Simultaneously, another sample of approximately 5 tons of the same cane was cleaned and then sampled by the core-sampler and

analyzed by the press method. The percent of cane trash, juice extraction, brix, fiber and sucrose was recorded for each cane delivery. The trash from the 5 ton samples was separated into tops and other trash.

RESULTS

Laboratory scale trial. Mixtures of tops and leaves caused a reduction in the normal juice extraction and sucrose % cane of the clean cane (Table 1, Figure 1). The juice extraction of clean cane was reduced by 30% at the 20% trash level. Sucrose reduction concentration of 5.7% on cane containing 5% of trash was observed in this experiment indicating the importance of trash in determining sugar recovery.

The trash percent cane was closely correlated with the (1-sucrose ratio) with $R^2=0.99$ (Figure 2). However, the slope according to the theoretical determination described above should be 100, whereas in this case a value of 61.6% was obtained.

Commercial Trial. The average composition of the trash in the commercial cane was: 65% leaves, 11.0 % tops and 24.0% other trash (suckers, soil, etc.). The trash content varied between 1.0% and 10.0 % during the experimental period (January - May).

The extraneous matter levels of the commercial cane were grouped in such a manner that all the samples between 0.5 and 1.5% trash were used to give a composite result with a trash level of 1% at intervals of one, between 1.5 and 2.5% for a composite sample with 2% etc. The trash content was highly correlated ($R^2=0.80$) with (1-sucrose ratio). The slope of 70 was reasonably close to the value of 61.6 obtained in the laboratory trial (Figure 2 & Figure 4).

DISCUSSION

The close correlation between the trash level and (1-sucrose ratio) and the similarity of the slope in the two trials indicate that this relation could be used to estimate the trash level in commercial cane samples using a modified core sampler procedure. This procedure can be envisaged as (a) a standard core sample taken from the wagon and (b) a small sample of cane taken from the wagon, cleaned manually and then subjected to the standard procedures for measuring sucrose in cane.

The theoretical basis of the sucrose ratio (Equation 6 above) indicates that the slope of the calibration curve should be 100, whereas we obtained values of 60-70. We surmise that this difference in the slope is due to sucrose in trash. This effect will be the subject of further studies.

The methodology also has an interesting aspect in that if there is sucrose in trash the estimate of trash will be reduced and we will obtain an *apparent trash* level. This leads to the possibility of using this methodology to determine an *apparent trash* level or in other words the estimate would compensate for the sucrose in the trash based on its contribution to the sugar in the commercial cane sample.

CONCLUSIONS

- Trash consisting only of green tops, leaves and mud reduced the values for both normal juice extraction and sucrose % cane; therefore the core/press method may be used for determining the percentage or level of trash in the sugarcane after harvesting.
- The sucrose % cane ratio of dirty to clean cane of the same post harvest sugarcane plot, based on core/press method showed that it was effective for determining trash levels.
- The statistical analysis of laboratory data from extracted juices by core/press method showed that 1.0 % of trash caused an average reduction of 1.0 % of sucrose % cane, but it was more significant at levels higher than 5 % of trash in clean cane.

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Table 1. Effect of cane trash quantity on milling quality of sugarcane, MZC74-275, CCSP89-1997 and CC91-1999 varieties.

Trash %	Fiber % cane	Extraction %	Sucrose % cane	ERS* %	Purity %	** (1-Ec/Eo)	*** (1-Sc/So)	Sucrose loss %	Estimated sugar loss
0	14.3	74.5	14.0	12.0	93.0	0	0	0	0
5	17.0	68.5	13.2	10.9	92.5	0.081	0.0571	5.7	9.2
10	20.1	62.5	12.0	9.4	91.7	0.158	0.1428	14.3	21.7
15	23.0	56.9	10.9	8.1	91.3	0.236	0.2214	22.1	32.5
20	26.6	50.8	9.8	6.7	90.7	0.318	0.3000	30.0	44.2

* ERS = estimated recoverable sugar

** Ec (%) = extraction of primary juice from dirty cane with a hydraulic press

Eo (%) = extraction of primary juice from clean cane with a hydraulic press

*** Sc (%) = sucrose % cane of dirty cane

So (%) = sucrose % cane of clean cane

Table 2. Effect of extraneous matter on juice extraction ratio and sucrose % cane ratio of dirty to clean sugarcane.

Extraneous matter range	Number of samples	* 1 - Ec/Eo	** 1 - Sc/So
0 - 2	16	-0.0025	0.0132
2 - 3	38	-0.00064	0.0047
3 - 4	12	-0.00157	0.0036
4 - 5	12	0.0216	0.0401
5 - 6	8	0.0202	0.0553
7 - 8	4	0.0226	0.0023
8 - 9	1	0.0341	0.1339
9 - 10	4	0.0311	0.1-37

* Ec/Eo = juice extraction ratio, dirty cane to clean cane

** Sc/So = sucrose ratio, dirty cane to clean cane

Table 3. Observed trash data from a sugar mill versus predicted trash using sucrose ratio of dirty to clean cane.

Test number	Observed trash (%)	* (1 - S_c/S_o)	** Predicted trash (%)
1	8.7	0.1178	8.2
2	8.8	0.137	9.6
3	6.8	0.1104	7.7
4	6.0	0.1037	7.2
5	3.2	0.047	3.3
6	4.6	0.0689	4.8
7	6.8	0.0963	6.7
8	5.7	0.0859	6.0
9	5.6	0.0815	5.7
10	2.6	0.0170	1.2

$$* \text{ Ratio} = \frac{S_c}{S_o} = \frac{\text{Sucrose \% cane of dirty cane}}{\text{Sucrose \% cane of clean cane}}$$

$$** \text{ Trash (\%)} = 70(1 - \frac{S_c}{S_o})$$

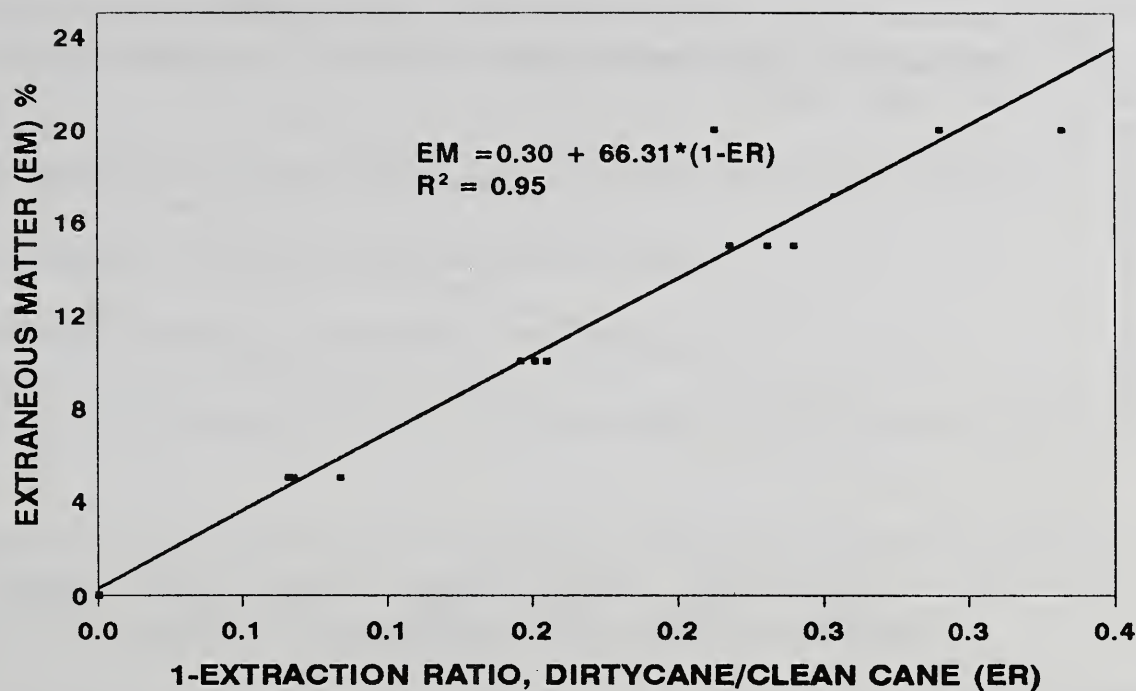


Figure 1. Juice extraction ratios, dirty cane to clean cane, for MZC74-275, CCSP89-1997 and CC91-1999 versus variations of extraneous matter.

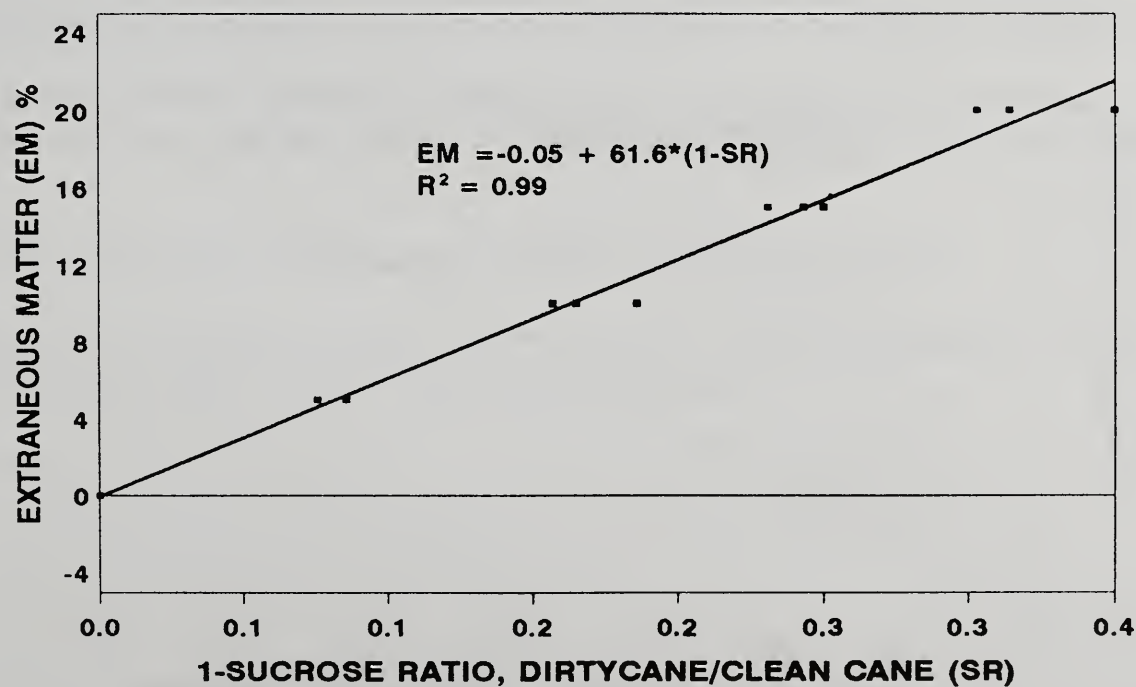


Figure 2. Sucrose ratios, dirty cane to clean cane, for MZC74-275, CCSP89-1997 and CC91-1999 varieties versus variation of extraneous matter.

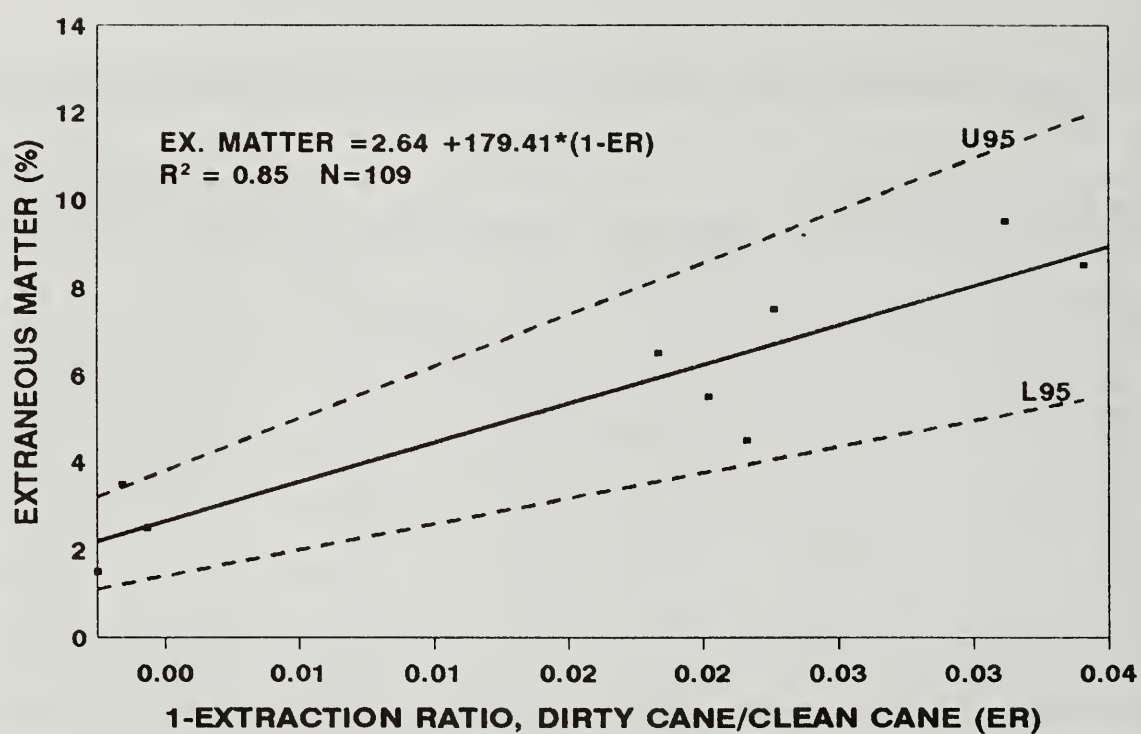


Figure 3. Prediction of extraneous matter using juice extraction ratios for commercial cane.

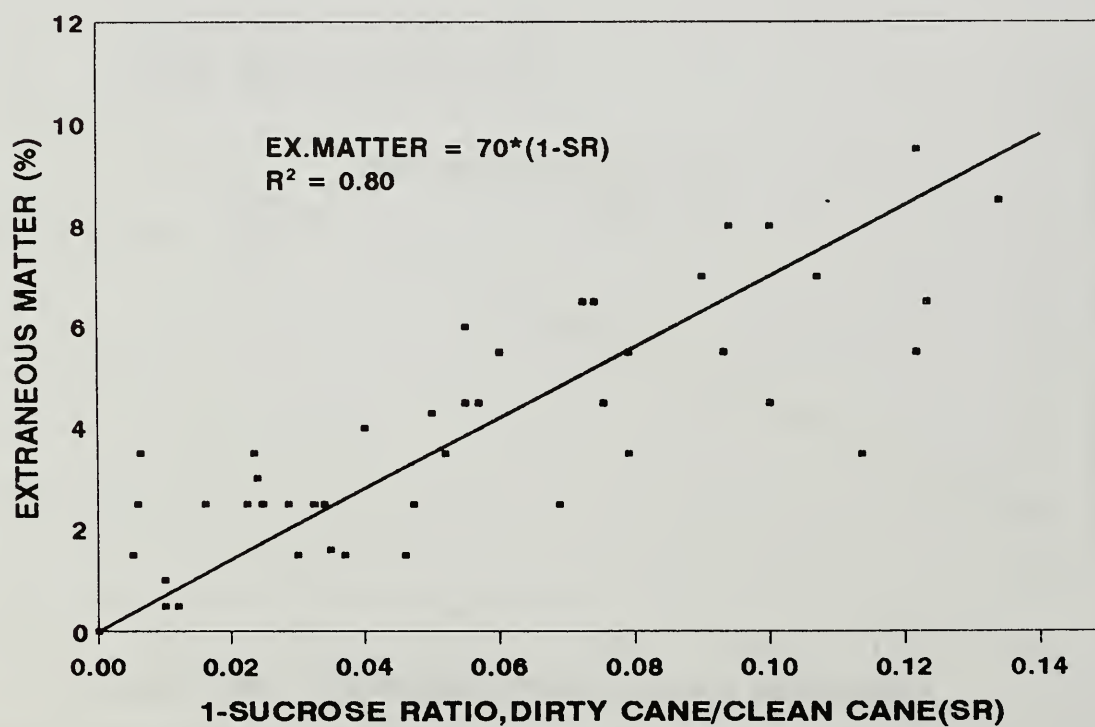


Figure 4. Prediction of extraneous matter using sucrose % cane ratio of dirty cane to clean cane.

DISCUSSION

Question: What is your definition of tops? Where do you start assuming it is a top, because there is a big difference between Florida and Colombia on where the top starts. This is especially relevant if you are using a combine.

Larrahondo: The topping height in Colombia is 30 cm below the natural breaking point.

Question: So the tops over there are more than they are here?

Larrahondo: Yes. Sometimes, the tops also contain sugar.

Question: Also, if you measure the Brix and it is lower than a certain value, it is considered tops, is that true?

Larrahondo: Yes. We analyzed the tops and find they contain some sucrose, but we are not too worried about this because in Colombia we are applying ripeners. The tendency is to try to top the cane a little higher than normal, especially when ripeners are used, to maximize tonnage.

Question: Where do you take the sample for the manual determination of trash? In your slide, it looked like it came from the top of the truck.

Larrahondo: For this trial, we used a grab from the top of the truck and we sampled different trucks.

Question: That gives you a different trash than the core sampler, so it is difficult to compare the two sets.

Larrahondo: Yes. We took grab samples randomly from 5 ton wagons and manually cleaned the cane. We will not continue to use this system for future studies because it is very time consuming and expensive.

Question: Have you looked at the influence of varieties on the amount of trash?

Larrahondo: Yes. Breeders are also trying to produce varieties with special characteristics for mechanical harvesting, which is being implemented in Colombia.

MARKER COMPOUNDS IN SUGAR INDUSTRY SAMPLES INDICATING SUGAR LOSS

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ABSTRACT

The most accurate determination of sugar losses in sugar manufacture and refining would be to analyze for a stable sugar degradation product, that is a marker compound. Under alkaline conditions, glucose, fructose, mannose and, to a much lesser extent, psicose are in initial equilibrium via a reversible isomerization reaction. Detection of mannose in samples taken from industrial processes where alkaline conditions prevail, confirmed that alkaline degradation of glucose and fructose (invert) had occurred. Mannose can be used as a marker compound for invert losses.

Oligosaccharides are formed in the breakdown of sucrose under acid and alkaline conditions, and have strong potential as possible stable markers. Potential oligosaccharide degradation product markers, formed across various sugar industry unit processes are reported and discussed, and compared to degradation products in model study solutions. The model study sucrose degradation reactions were undertaken under simulated industrial conditions (65 °Brix; constant pH 5.45-9.25; N₂; 100 °C).

Improved ion chromatography technologies for separation and fractionation of sugar loss markers are discussed, as well as the use of electrospray mass spectrometry to aid the identification of markers.

INTRODUCTION

Although various workers have made meaningful attempts to measure accurately the sucrose losses which occur in unit process operations in sugar manufacture and refining, such losses have been very difficult to measure. This is because sucrose usually occurs in relatively higher concentrations in industrial samples compared to invert sugars. Losses in evaporation (1, 2, 3,4) and clarification (5,6) processes have been particularly difficult to measure, even with the modern techniques of gas chromatography and ion chromatography with integrated pulsed amperometric detection IC-IPAD (also known as high performance anion-exchange chromatography HPAE-PAD). Since actual sucrose loss may be smaller than the analytical error, markers have been used to estimate loss, and glucose has been used often as a marker. However, glucose is also destroyed in many of these processes, and the exact amount destroyed is not known. Therefore, as a marker it offers only minimum loss values which are underestimates.

The most accurate determination of sucrose loss would be to analyze for a stable sugar degradation product, i.e., a marker compound, and this compound will differ among various industrial processes, because of the varying pH conditions which induce either acid or alkaline sugar degradation reactions (7).

We (5,7) have previously reported possible oligosaccharide (short chain sugars) markers in various unit processes, that were detected with ion chromatography using a strong NaOH/NaOAc gradient method. This paper reports the status of an ongoing study to detect and identify markers which could be used to determine sugar degradation and losses across sugar industrial unit processes.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade sodium hydroxide (50% solution) and sodium acetate were obtained from Fisher Scientific. Millipore water (resistance of 18 M Ω) was used to prepare eluents and samples. Prepared eluents were sparged with high purity helium to remove carbonate.

Standards

All sugar standards were analytical grade. Fructose, raffinose, stachyose and mannose were obtained from Aldrich, sucrose, glucosamine-HCl and glucose from J.T. Baker, myo-inositol from Calbiochem-Behring and psicose from Sigma.

IC-IPAD Analysis of Sucrose, Glucose and Fructose: Gradient Method

The IC-IPAD equipment used was a Dionex (Sunnyvale, CA) BioLC instrument. Sucrose, glucose and fructose were separated on Dionex CarboPac PA-1 guard (25 x 4 mm) and analytical (250 x 4 mm) columns, at a flow rate of 1.0 mL/min at ambient temperature (~25 °C). Column eluent conditions were: 16 mM NaOH isocratic (inject; 0.0-2.0 min), a gradient of 16-160 mM NaOH (2.0-35.0 min), followed by isocratic 200 mM NaOH (35.1-40.0 min), and return to 16 mM NaOH (40.0-49.0 min) to re-equilibrate the column with the initial mobile phase prior to the next sample injection. Carbohydrates (25 μ L injections) were detected using integrated pulsed amperometric detection (IPAD). The detector was equipped with Au working and Ag/AgCl reference electrodes, operating with the following working electrode pulse potentials and durations: $E_1=+0.05$ V ($t_0=0.00$ s), $E_2=0.05$ V ($t_1=0.42$ s), $E_3=+0.75$ V ($t_3=0.43$ s), $E_4=+0.75$ V ($t_4=0.60$ s), $E_5=-0.60$ V ($t_5=0.61$ s), $E_6=-0.60$ V ($t_6=0.96$ s). The duration of the IPAD integration interval was set at 0.2-0.4 s. Using a Spectra-Physics SP8880 autosampler, refrigerated at 4 °C, and Dionex PeakNet 4.30 chromatography software, runs were accumulated of multiple samples and standards, with a blank being first run to stabilize column and system performance. The standards were myo-inositol, glucosamineHCl (internal standard), glucose, fructose, sucrose, raffinose and stachyose. Seven different levels of the standards were run first, and standard curves were generated (sucrose ranged from 1 to 25 ppm) to test linearity in multiple runs and generate area response factors. Weight diluted samples were run in duplicate. Glucose and fructose were quantified in different runs than sucrose, due to the very different concentrations of these carbohydrates in the samples. There was a much larger dilution for sucrose quantification. Response factors were generated for each of the carbohydrates, using internal standard calibrations and check standards.

IC-IPAD Analysis of Oligosaccharides: Gradient Method

Oligosaccharides (mainly 2 to 12 degree of polymerization) were separated on Dionex CarboPac PA-1 guard and analytical anion exchange columns (250 x 4 mm), at ambient temperature (~25 °C). Flow rate = 1.0 mL/min. Diluted industrial sugar samples were filtered through 0.45 µm filters. Eluent conditions were: 100 mM NaOH isocratic (0.0-1.0 min; inject 1.0 min), a gradient of 0 to 300 mM NaOAc in 100 mM NaOH (1.1-40.0 min), and return to 100 mM NaOH (40.1-45.0 min) to re-equilibrate the column. Oligosaccharides (from 100 µL injections) were detected using integrated pulsed amperometric detection (IPAD) and detector settings were the same as for the sucrose, glucose and fructose gradient method. Using a Spectra-Physics SP8880 autoinjector and Dionex Peaknet chromatography software, runs were accumulated of multiple samples and standards.

IC-IPAD Analysis of Mannose: Isocratic Method

Mannose was separated on Dionex CarboPac PA-1 guard and analytical anion exchange columns (250 x 4 mm), at ambient temperature (~25 °C). Eluent conditions were : 8mM NaOH isocratic (0.0-20.0 min; inject 1.0 min), followed by isocratic 200 mM NaOH (20.1-25.0 min) and return to 8 mM NaOH (25.0-30.0min) for re-equilibration. Diluted industrial sugar samples were filtered through a 0.45 µm filter and a 25 µL injection volume was used.

°Brix

°Brix was measured using a Leica Abbe Mark II Refractometer with a crosshair reticule, and is the average of at least three measurements.

Fractionation of Marker Compound IC-IPAD Peaks

IC-IPAD peaks were fractionated using an ISCO Foxy Junior™ automated fraction collector, using retention time and peak slope detection. Sodium ions (Na⁺) were removed from the samples and eluent after cell detection, by placing a Dionex carbohydrate membrane desalter (CMD™) between the detector cell and fraction collector. A self-regenerating suppressor controller was used and the pneumatically supplied regenerant was 75mM H₂SO₄. Fractionated samples were freeze-dried, re-dissolved in 0.5 mL of water, filtered through 0.45 µm filters to remove any dust particles, and then freeze-dried again.

Electrospray Mass Spectrometry

Mass spectra were recorded on a Finnigan MAT (San Jose, CA) LCQ equipped with an electrospray ionization source. Electrospray ionization was performed under the following conditions. The spray voltage was set at 3.7 kV. The sheath gas consisted of nitrogen gas (99.5%) and was set at 60 units. The

heated capillary temperature was set at 250 °C, and the capillary voltage was set at 0 V. The ion injection time was 5 ms. The fractionated, freeze-dried samples were dissolved in 50% methanol (500 μ L) and injected by direct infusion at a flow rate of 30 μ L/min. A full scan was recorded over a range of 50 - 2000 u with 3 micro scans for a 0.25 min duration. This was followed by a 100 - 900 u scan for a 0.25 min duration. A third segment was programmed with three events: a 50 - 500 u scan, a zoom scan on the most intense ion, and a MS/MS on the most intense ion with a 2 u isolation width and 29 % relative collision energy.

Constant pH Model Systems

Constant pH reactions were undertaken using an autotitrator. See Eggleston (8) for full method.

RESULTS AND DISCUSSION

Mannose as a Marker Compound to Determine the Alkaline Induced Degradation of Invert

Under alkaline conditions, glucose, fructose, mannose and, to a much lesser extent, psicose are in initial equilibrium via an enediol anion intermediate, which subsequently undergoes further non-reversible degradation reactions and subsequent color formation. The mechanism of this reversible isomerization is known as the Lobry de Bruyn-Alberda van Ekenstein rearrangement (9). The presence and formation of mannose in an industrial sugar unit process would, therefore, confirm that alkaline degradation of invert had occurred. Although color formation is also a strong indicator that alkaline degradation of invert has occurred, many different reactions (10) contribute to color formation in alkaline industrial sugar streams, whereas the presence of mannose as a marker compound is a more specific confirmation test. Using a gradient NaOH eluent IC-IPAD method, Eggleston (4) recently observed that mannose was present in thin juice and evaporator syrup samples, collected across a sugarbeet factory's evaporation process, which is shown in Figure 1. The mannose concentration was observed to increase across the process, and the alkaline degradation of invert was shown to be more dominant at the later evaporation stages (4). Figure 2 shows that mannose was also found to be present in juice samples collected from a sugarbeet factory's sweet water tank, after milk of lime had been added.

Development of a New IC-IPAD Method to Separate Mannose from Glucose

As shown in Figures 1 and 2, using a gradient NaOH eluent IC-IPAD method, mannose is eluted as a shoulder on the tail of the glucose peak, which interferes with identification and quantification of mannose. Consequently, a new IC-IPAD method was developed to improve the separation of mannose from glucose. Reducing the eluent concentration to 8mM NaOH and running the eluent isocratically, resulted in an acceptable separation of mannose from glucose. Results from this new isocratic method are shown in Figure 3. Furthermore, results (unpublished) from this laboratory showed that the mannose peak is not an interfering amino-acid peak.

Oligosaccharides as Marker Sugar Loss Compounds

Oligosaccharides are formed in the breakdown of sucrose under both acid and alkaline conditions, and have strong potential as possible stable marker compounds, to accurately determine sucrose losses across various unit processes. Eggleston (8) developed a strong sodium acetate/sodium hydroxide gradient IC-IPAD method, to separate oligosaccharides (with up to 12 monosaccharide units or degree of polymerization, DP) in juices and concentrated sugar liquors found in the sugar industry (4-7). This method allows monitoring of numerous samples with minimal sample preparation. Furthermore, the anion-exchange column used (Dionex CarboPac PA-1TM) can tolerate high overload levels of sucrose and still detect low levels of oligosaccharides, without requiring extensive column washings between runs. Oligosaccharides formed across three different processes from the sugarcane and sugar beet industries, were recently reported by Eggleston et al (7).

An investigation of sugar losses in the “cold lime” clarification of mixed juice from fresh and “stale” (freeze deteriorated) sugarcane was undertaken. Using the sodium acetate/sodium hydroxide gradient IC-IPAD method, the oligosaccharides formed and destroyed across the clarification process, as well as across the grinding season, were monitored and select results are illustrated in Figures 4a,b,c. Figures 4a and 4b show the oligosaccharide chromatograms of mixed (MJ), limed (LJ), heated limed (HLJ) and clarified juice (CJ) from fresh (4a,b) and stale, acidic (4c) cane, respectively. Extensive amounts of oligosaccharides were present in all samples, especially the stale cane. Oligosaccharides present in the MJ could have originated from the field cane, from sugar degradation reactions that occurred across the mill end of the factory, and also because of formation of dextran oligosaccharides (7). In Figures 4a and b, where mixed juice from fresh cane was clarified at the middle, and end of the cane grinding season, respectively, an intense peak was clearly visible (Retention time $R_t = \sim 32$ min) in the limed juice but not in the other samples. This indicates strongly that it was an alkaline sugar degradation product which formed in the lime tank. This peak, as shown in Figure 4a, has been denoted “X” and was fractionated for further electrospray mass spectroscopy (ESMS) studies, which is discussed in the “Fractionation” section below.

In comparison, in Figure 4c, where mixed juice from stale cane (field cane subjected to freezing then warm weather conditions) was clarified, acid degradation reactions occurred (6) in the lime tank, because of the very acidic, stale cane, poor mixing of lime and a reduction in residence time (6). Although an intense peak ($R_t = \sim 32$ min) was present in the mixed juice, this was destroyed, not formed in the lime tank. This is further evidence that peak “X” in Figures 4a and b, is a product of the alkaline degradation of sucrose and/or invert.

Fractionation of Sugar Loss Marker Compounds

Qualitative and quantitative comparisons of IC-IPAD chromatograms of samples across unit processes, can indicate the increased formation of stable sugar degradation product peaks, which could be used as marker compounds. The next steps are to fractionate, and then identify the peak compound(s). We have very recently begun to use electrospray mass spectrometry (ESMS) to aid in the identification of such markers. IC-IPAD uses the alkaline eluent, sodium hydroxide (NaOH), and for oligosaccharide analysis,

sodium acetate (NaOAc) is also used. However, we observed that peaks fractionated in NaOH solutions are very liable to undergo alkaline degradation reactions on collection, and particularly on freeze-drying, where not only are the collected peaks concentrated but the eluent compounds as well. Furthermore, mass spectroscopy cannot be undertaken if numerous Na^+ ions are present, as they would overload the spectrometer and create a large and unwanted background signal. It is, therefore, necessary to neutralize, and remove Na^+ ions, from the eluent after IC-IPAD detection and before fraction collection.

We have been able to remove Na^+ ions from the sodium hydroxide and/or sodium acetate eluents after passage through the columns and detector cell, by using a Dionex carbohydrate membrane desalter (CMDTM). The CMD was employed between the detector cell and fraction collector, and this is illustrated in Figure 5. The CMD uses a counter-current cation exchange mechanism, where H^+ ions are exchanged for Na^+ ions. This is driven by both electrolytically generated and pneumatically supplied H_2SO_4 regenerant. Sugar degradation products eluted in NaOH, elute from the CMD in water; if the eluent contains NaOAc, they elute in weak acetic acid (HOAc). As HOAc is volatile, it is removed on freeze-drying. We have routinely observed that the eluent is reduced from $\sim \text{pH } 13$ to 6.5 .

Dispersion of the fractionated peaks in the post-detection tubing and the CMD, is minimal and the signal at the fraction collector is very similar to the signal at the detector cell (11).

Identification of Sugar Loss Marker Compounds - Use of Electrospray Mass Spectrometry

In order to ascertain which method of ionization was the most appropriate for studying the electrospray mass spectra of simple sugars and oligosaccharides, a preliminary ESMS experiment was first undertaken on a psicose (monosaccharide) standard. Positive ionization gave the most informed results and we, consequently, used this method of ionization in subsequent ESMS analysis of fractionated IC-IPAD peaks.

As discussed in the section titled "Oligosaccharides as Marker Sugar Loss Compounds", a possible marker compound "X" was fractionated from limed juice, that had undergone alkaline degradation (see Figure 4a) and the ESMS results for "X" are shown in Figure 6. The most intense MS peak occurred at m/z 241.5 (see Figure 6a) and a zoom scan in Figure 6b shows, from the spacing between the ^{12}C and ^{13}C isotope peaks, that it had a double positive charge, which indicated that the actual compound was 480.6u (u is equivalent to daltons). A further MS/MS (or MS^2) scan of the m/z 241.5 ion is shown in Figure 6c, and it is clear that this compound fragmented into characteristic 342u and 123u daughter ions. The 342u daughter ion has a characteristic molecular weight of a disaccharide, which suggests that the parent compound had a disaccharide "backbone" structure. It is, therefore, possible that the "X" compound is a condensation product, formed from the condensation of a disaccharide with a reactive reductone-type molecule. Another possibility is that "X" is a trisaccharide, although 480.6u is a low molecular weight for a trisaccharide. "X" may also be a high molecular weight acid, as De Bruijn (12) reported that substantial amounts of $>\text{C}_6$ acids are formed from the irreversible reactions of alkaline degradation of invert, after isomerization reactions initially occur. Furthermore, it could also be possible that "X" is a complex of three calcium (Ca^{2+}) ions, a sucrose molecule and a water molecule (13), although this is unlikely as the carbohydrate membrane desalter (CMD) removes all cations (Dionex, personal communication) and

would have been expected to remove most Ca^{2+} ions present in the sample. Further studies using nuclear magnetic resonance (NMR), Infra Red (IR) and Ultra Violet (UV) spectroscopy studies may further elucidate the structure of the fractionated "X" marker.

In our previous work (5,7) we favorably compared oligosaccharide chromatograms of industrial samples to those of model 65 °Brix sucrose degraded samples. The model samples had been reacted at 100 °C up to 8h under constant pH conditions (8), to simulate the various industrial process conditions. Such model study samples are very useful in the indication of the major sugar degradation products, their rates of formation and stability, which are all important criteria for their use as markers. Figure 7 illustrates a direct comparison between a dilute sweet water (DSW) sample from a mud desweetening system attached to the TALOTM clarification process of a sugarcane refinery, with a model sample that was reacted at constant pH 7.5. The most acid degradation of sucrose, across the refinery clarification process, occurred in the mud desweetening system (5,7).

Figure 7 shows that many similar peaks were present in the refinery DSW and model samples, which could be possible sugar loss markers. Peak "Y1" in the DSW sample and the corresponding similarly retained model sample peak "Y2" were fractionated, and electrospray mass spectra results of the fractionated peaks are shown in Figures 8 and 9, respectively. Numerous MS peaks were present in the full scan of the DSW "Y1" peak (see Figure 8) and the model sample "Y2" peak (see Figure 9), which strongly suggests that both peaks were impure. Although obvious differences in intense peaks are evident, similar MS peaks were also apparent. For example, the 263, 382.9 and 404.9u ions were all visible in both samples. This suggests that the "Y1" and "Y2" compounds are possibly the same and warrant even further structural investigation. However, it is also possible that the similar peaks are artifacts from the chromatography system or from the preparation of the freeze-dried sample. Further work is, therefore, necessary.

MAJOR CONCLUSIONS

- * Mannose was detected in various industrial sugar samples, and can be used as a marker to confirm that alkaline degradation of invert has occurred
- * A new IC-IPAD isocratic 8 mM NaOH method has been developed to separate better mannose from glucose
- * A fractionation system has been developed, using a carbohydrate membrane desalter (CMDTM), to remove sodium (Na^+) ions from the eluent and the sample. This allows freeze-dried fractionated peaks to be identified using electrospray mass spectroscopy.
- * Positive ionization mode of ESMS gave the best mass spectra results for simple sugars and oligosaccharides

* A 481 molecular weight marker has been observed in the limed juice of a sugarcane factory, where alkaline degradation reactions prevailed.

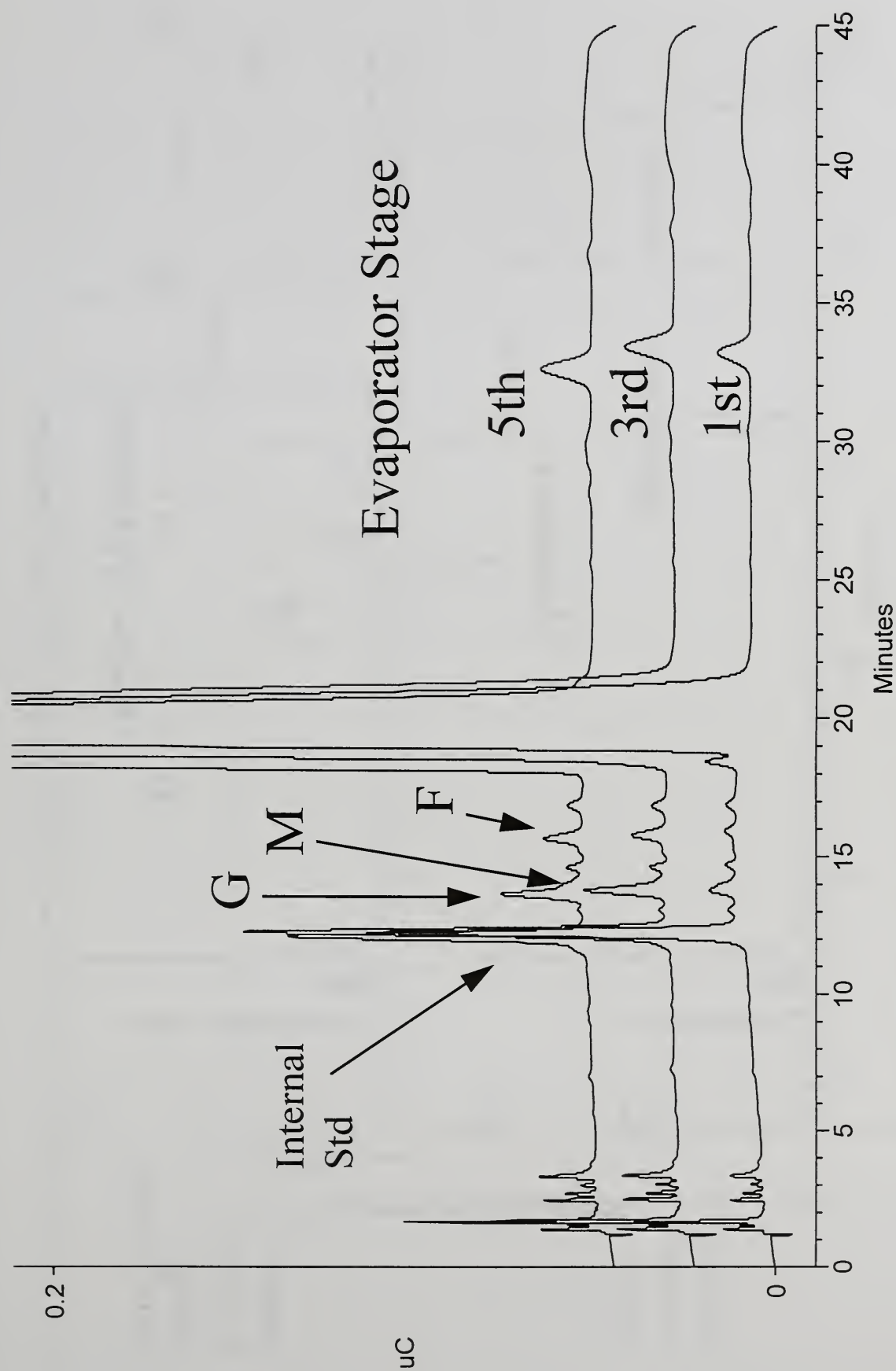
ACKNOWLEDGEMENTS

The authors thank Mr. Eldwin St. Cyr for excellent technical assistance, and to Drs. Casey Grimm and John Vercellotti for their useful discussions.

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^a From Eggleston (1998) (4)

Figure 1. Mannose (M) formation across the evaporation process of a sugarbeet factory (from Eggleston, 1998) (4).

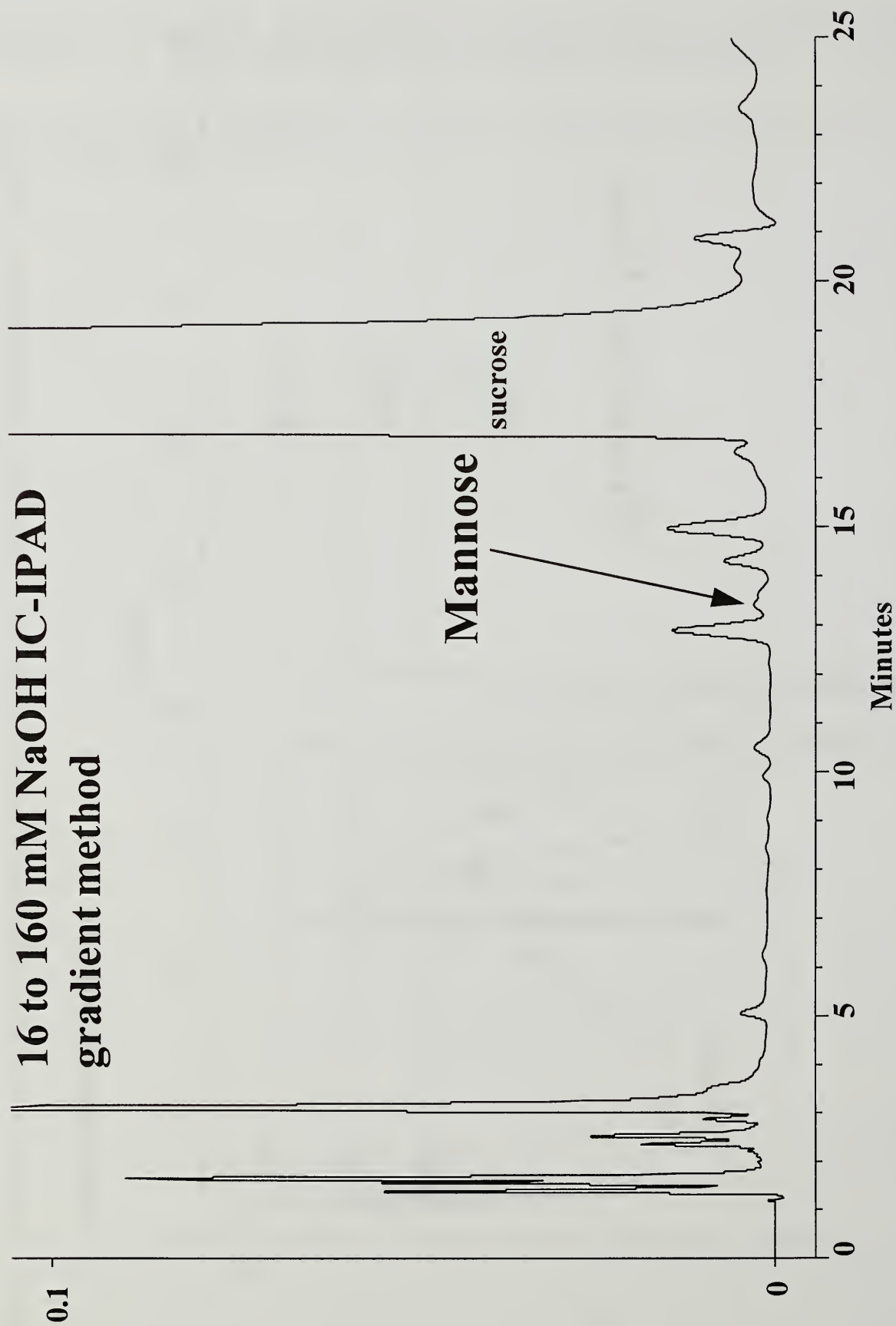


Figure 2. The presence of mannose in a sweet water tank juice, with milk of lime added.

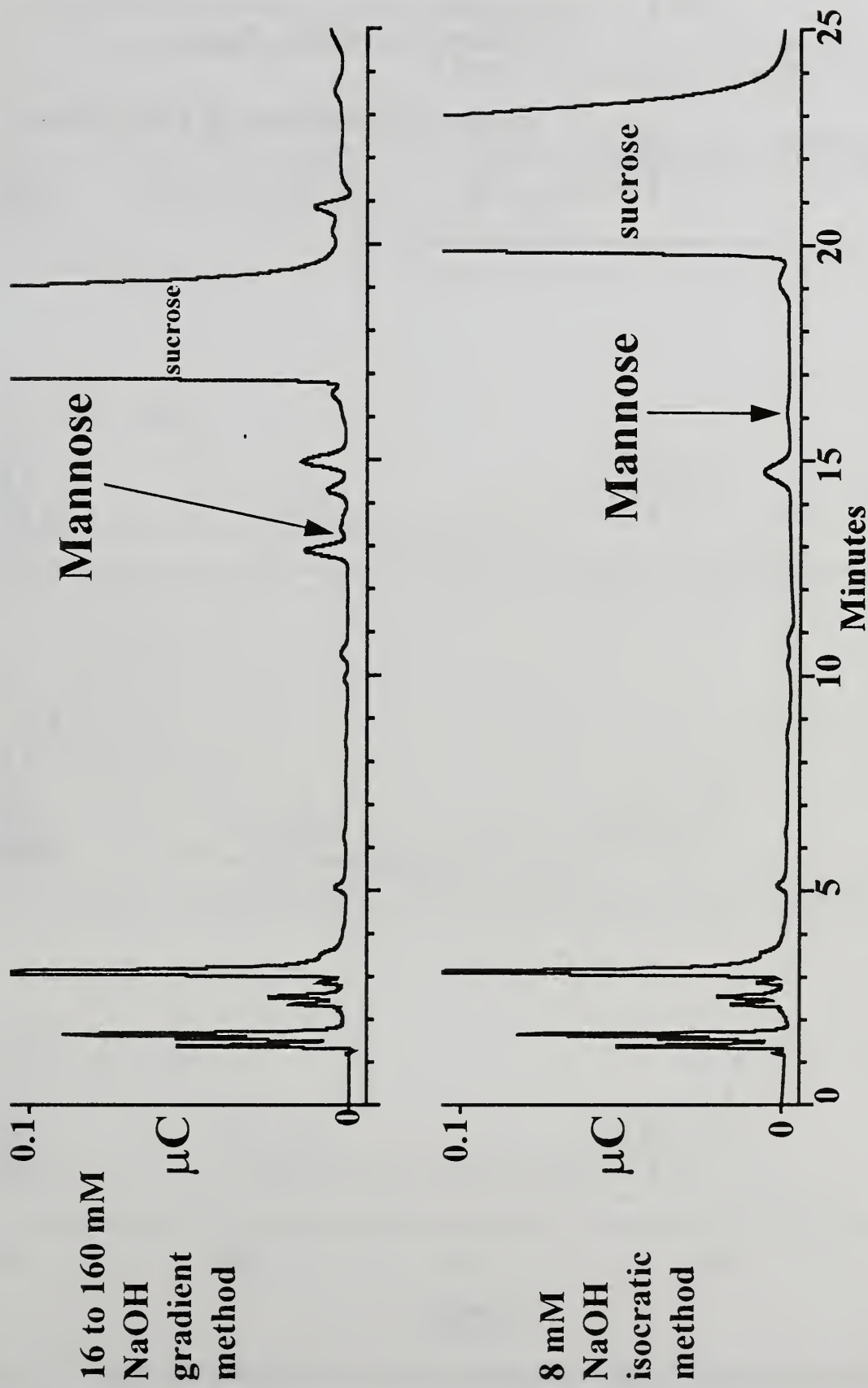


Figure 3. Improved isocratic IC-IPAD method for determining mannose. Sweet water juice samples with milk of lime added.

All samples = 11.2 Brix

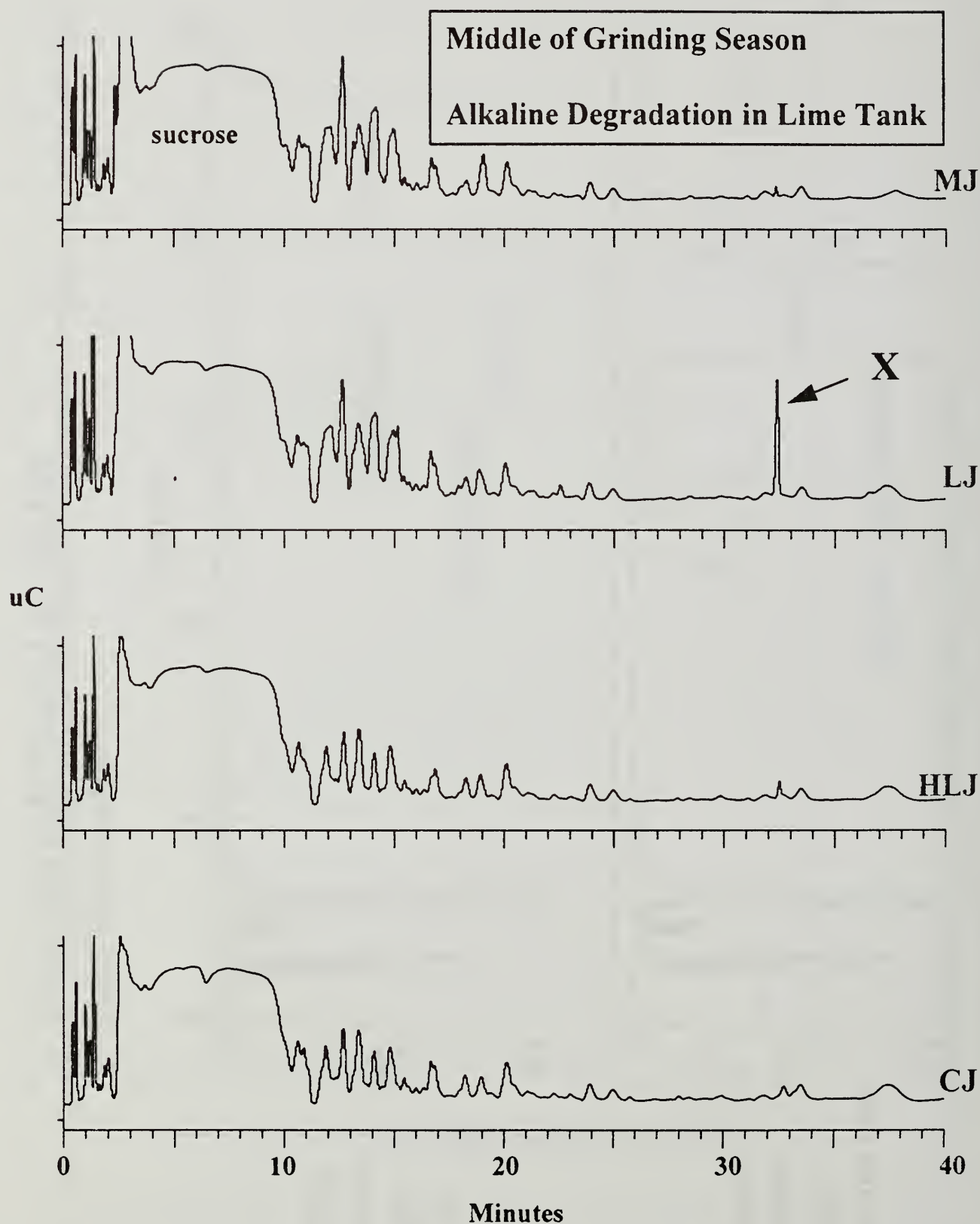


Figure 4a. IC-IPAD oligosaccharide formation across the lime clarification process of a sugarcane factory. Fresh cane. X = alkaline sugar degradation product.

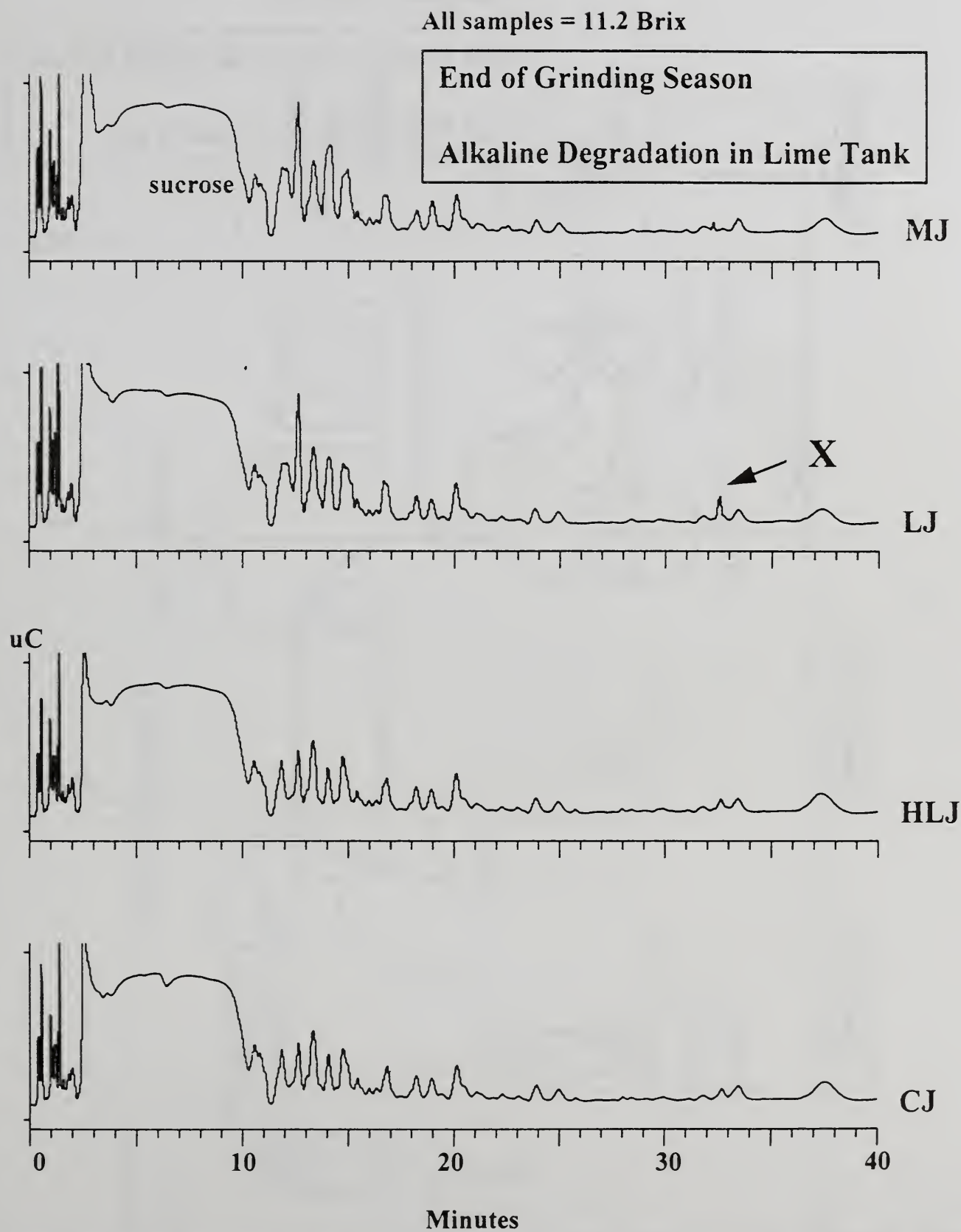


Figure 4b. IC-IPAD oligosaccharide formation across the lime clarification process of a sugarcane factory. Fresh cane. X = alkaline sugar degradation product.

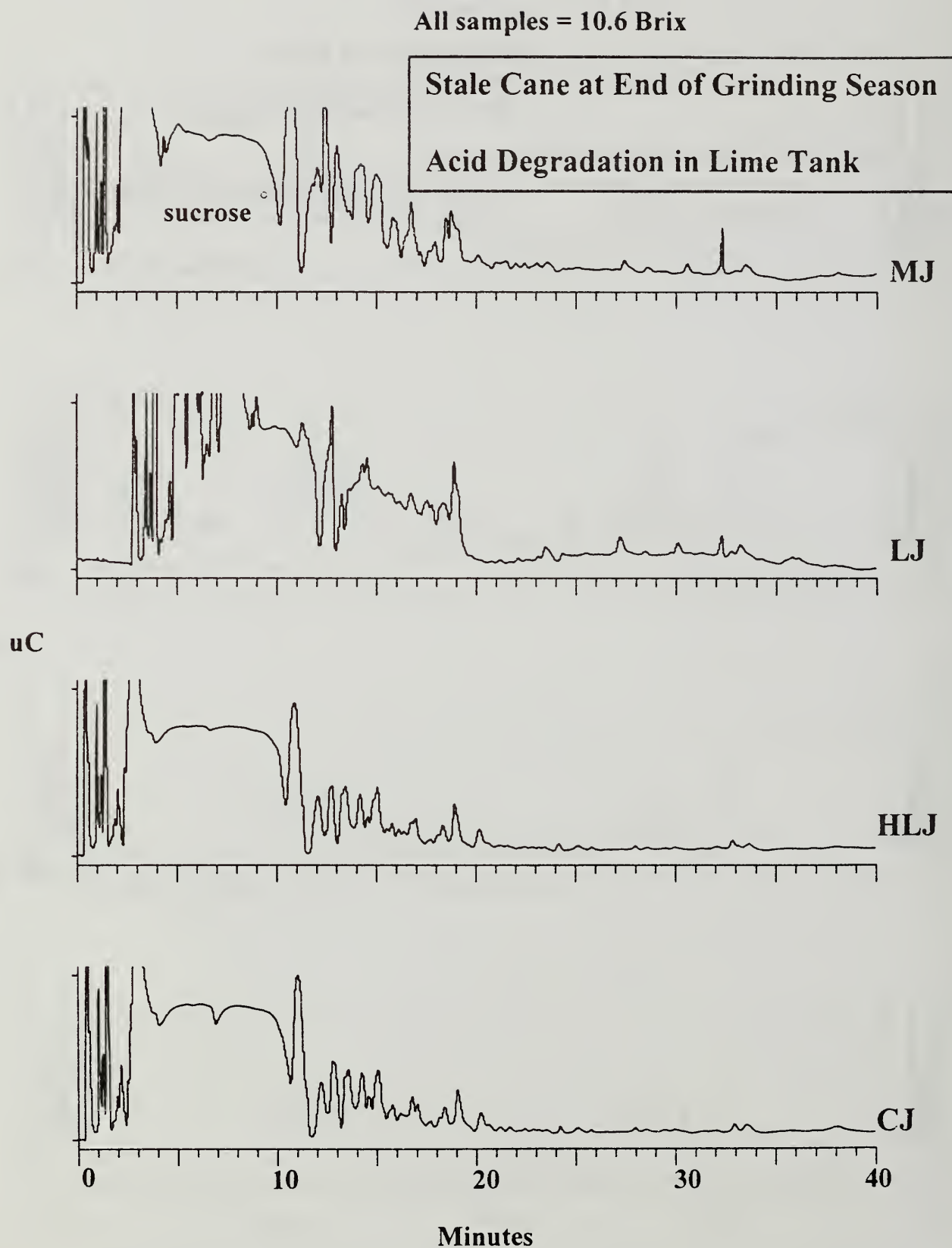


Figure 4c. IC-IPAD oligosaccharide formation across the lime clarification process of a sugarcane factory. Stale and acid cane, freeze damaged.

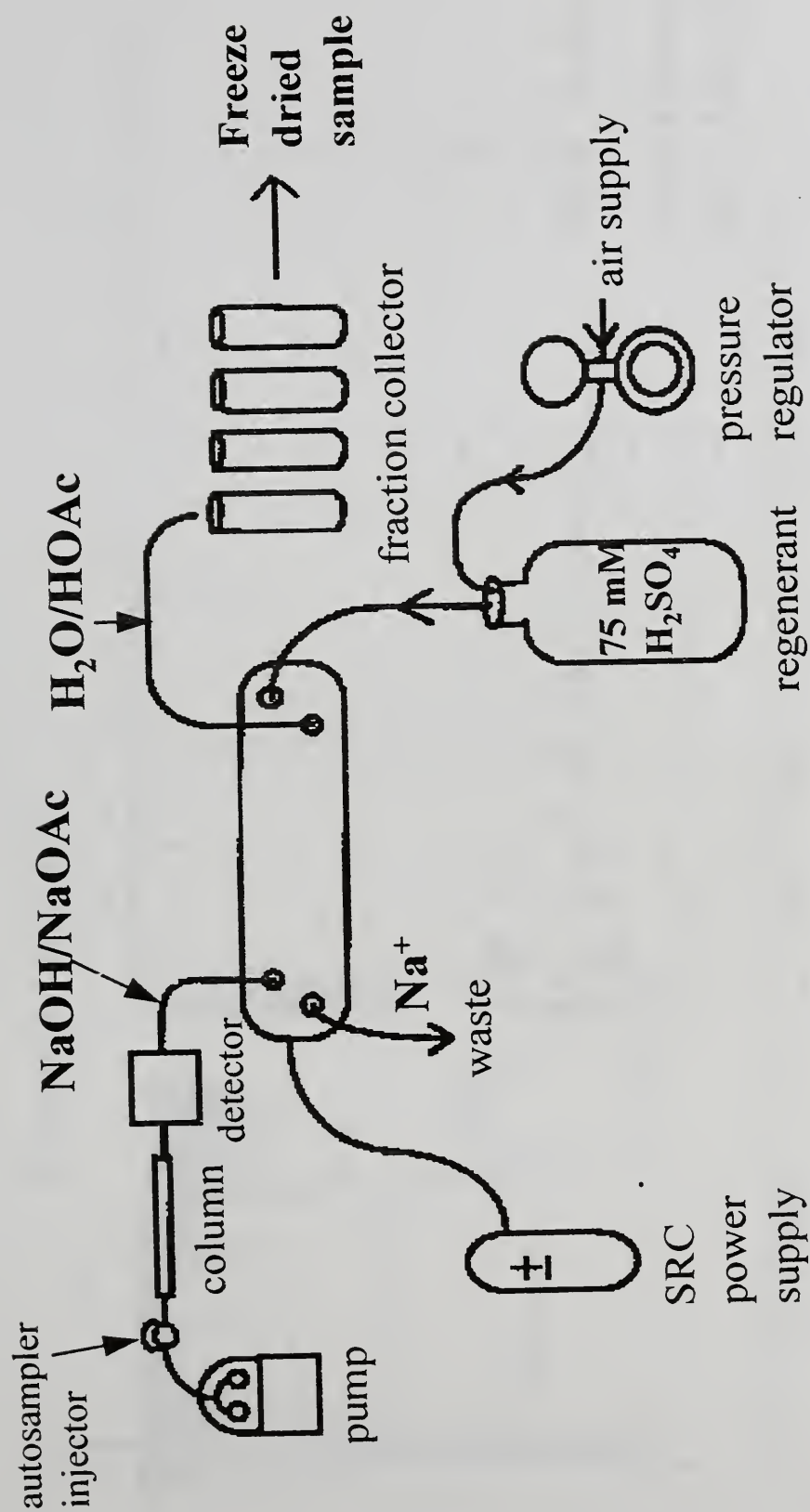


Figure 5. Fractionation of IC-IPAD peaks in desalted eluent using a carbohydrate membrane desalter (CMD).

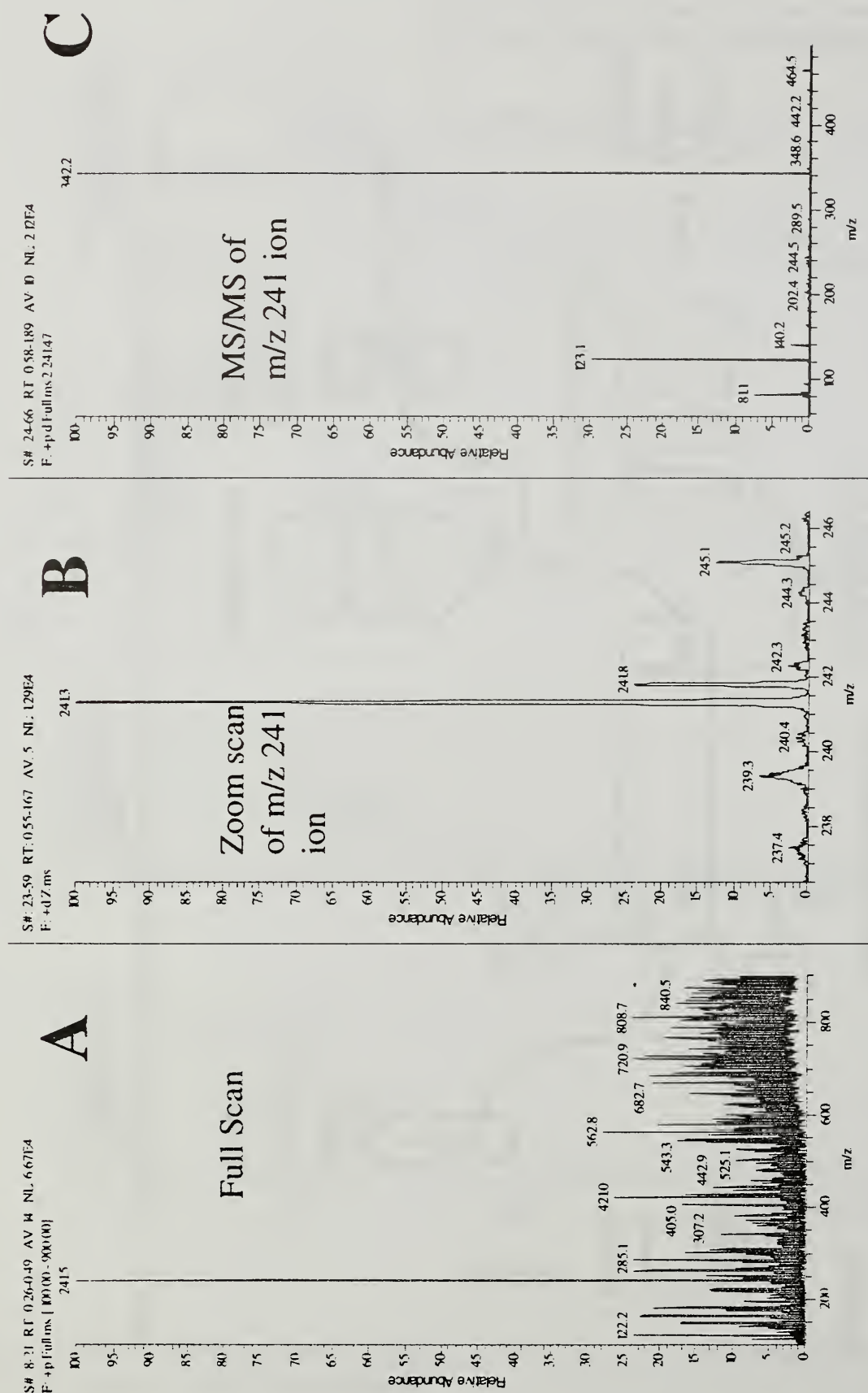


Figure 6. Electrospray mass spectrum of marker compound "X" (see Figure 4).

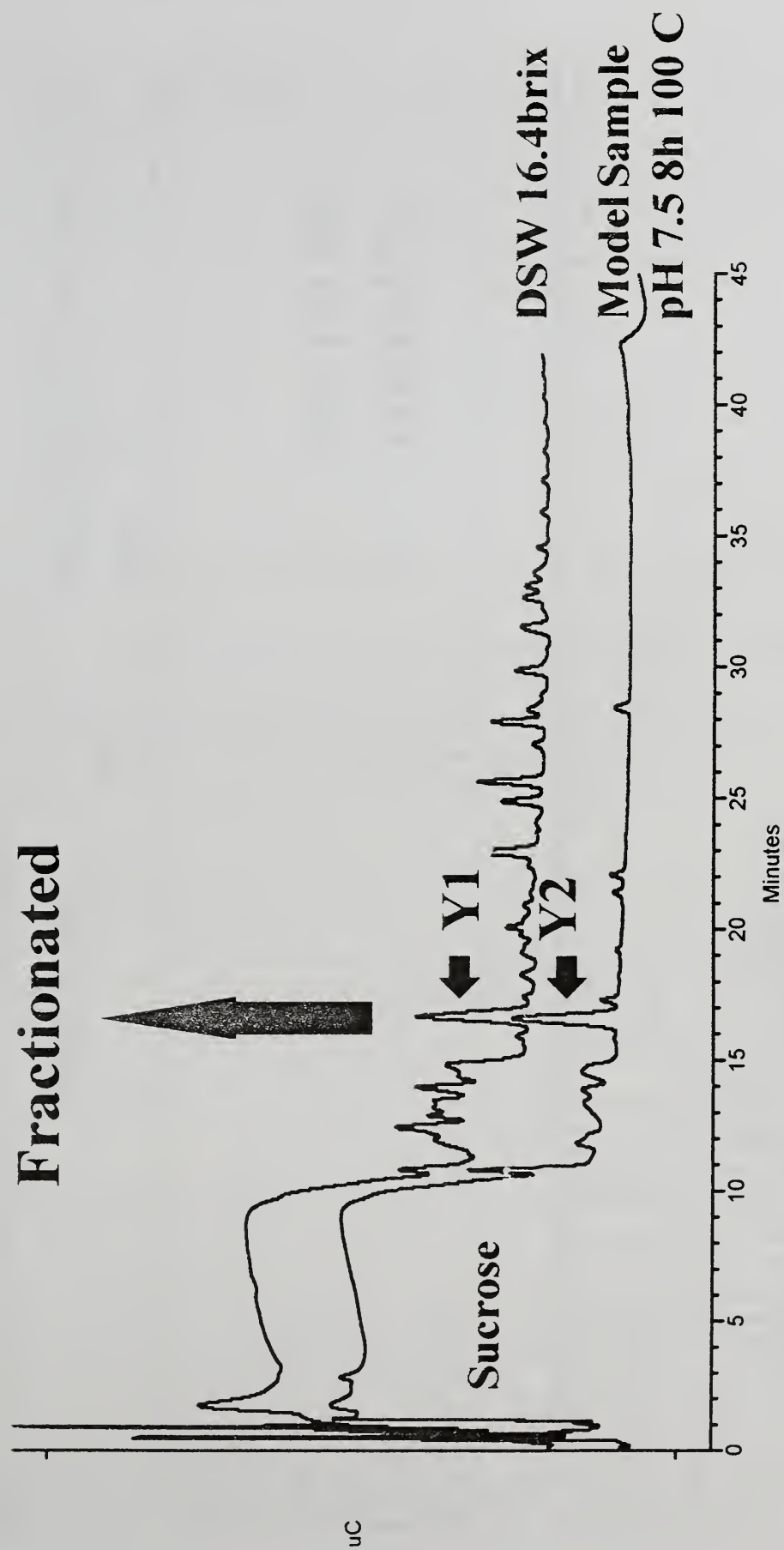


Figure 7. IC-IPAD oligosaccharide chromatograms: Fractionation of Y1 and Y2 peaks.

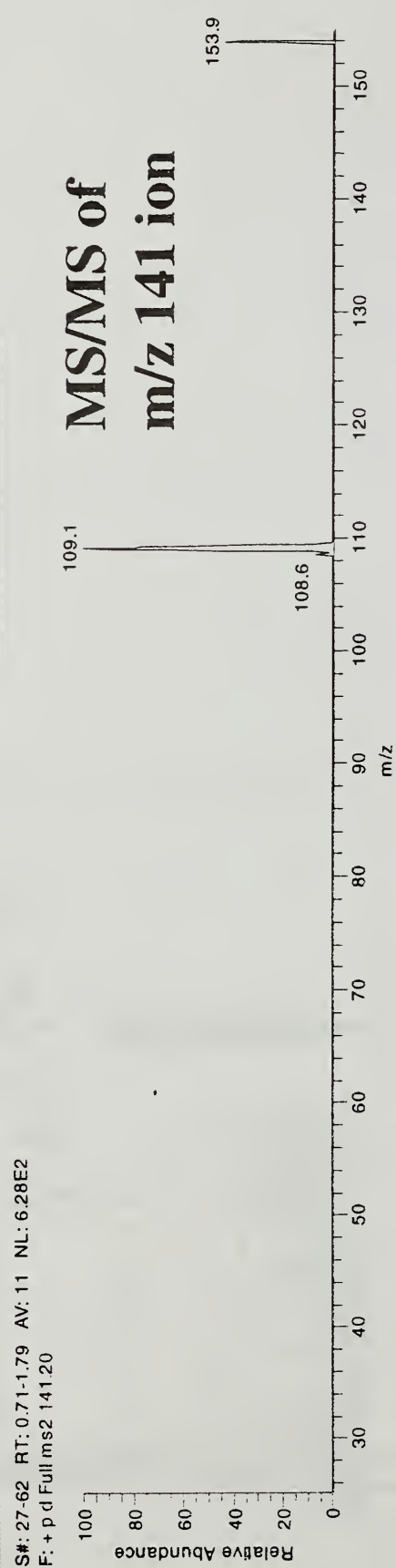
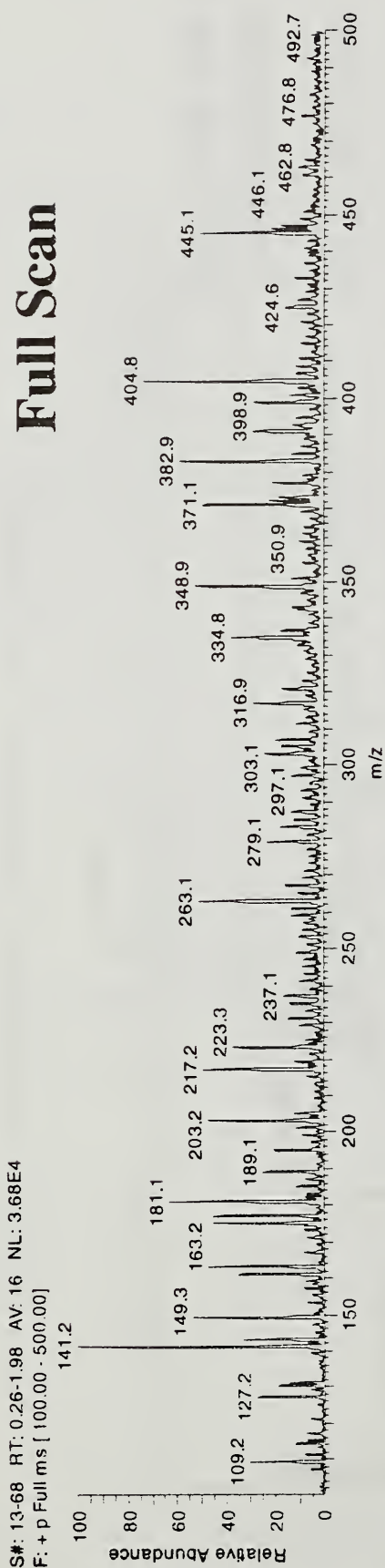


Figure 8. Electrospray mass spectrum of "Y1" (see Figure 7).

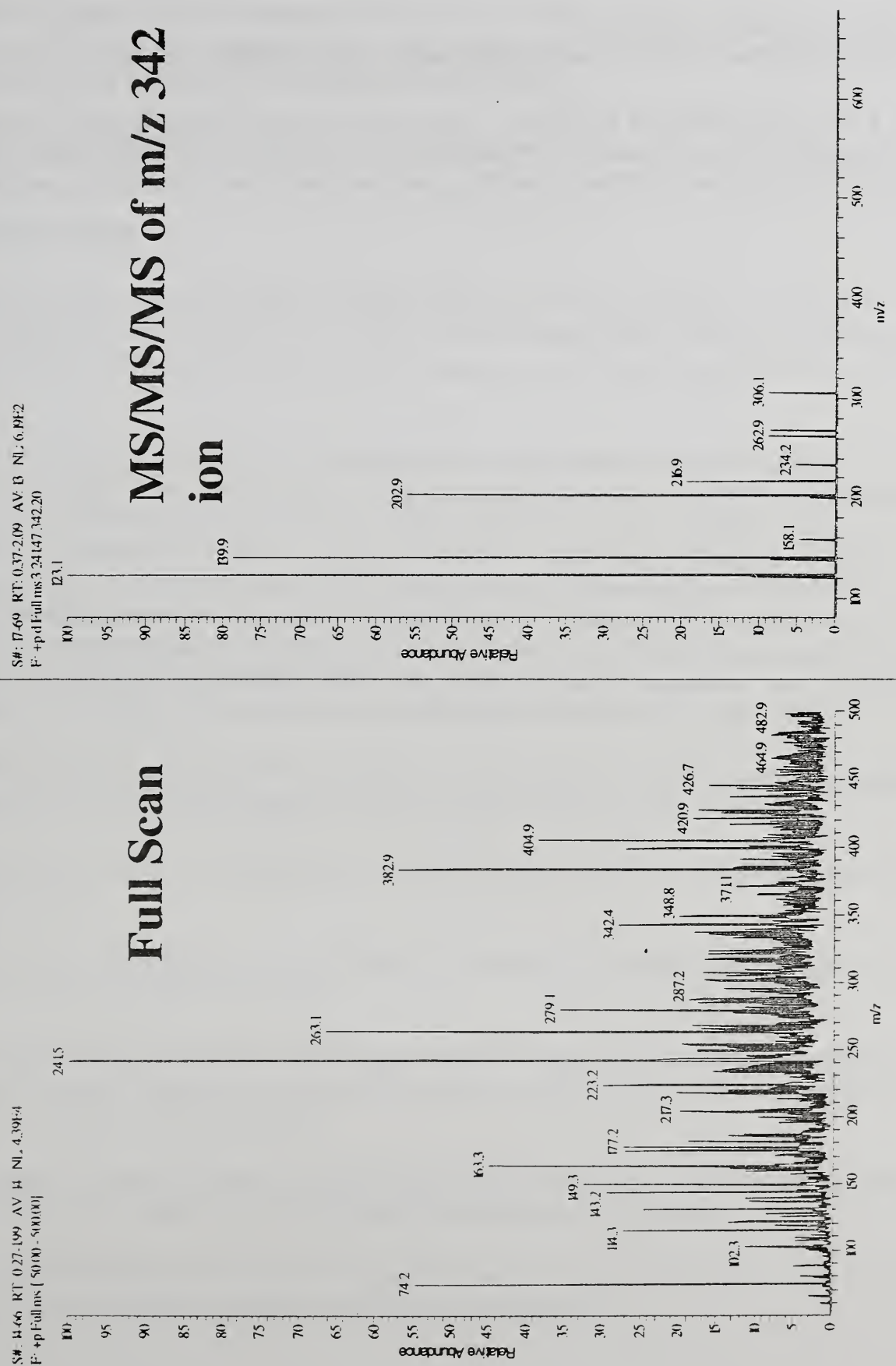


Figure 9. Electrospray mass spectrum of "Y2" (see Figure 7).

S.P.R.I.

DISCUSSION

Question: Is it possible that the compound with 400 molecular weight is calcium succrate?

Eggleston: Yes, I did mention that as a possibility. When you are trying to identify these compounds, you really need two methods of identification. Consequently, we still have further identification work to conduct although the carbohydrate membrane desalter system removes all cations, including calcium.

STUDY OF HMW COMPOUNDS IN SUGAR LIQUORS FROM CARBONATATION AND ION-EXCHANGE RESINS USING GEL PERMEATION CHROMATOGRAPHY WITH AN EVAPORATIVE LIGHT SCATTERING DETECTOR

Luis San Miguel Bento and Susana Sá, RAR-Refinarias de Açúcar Reunidas, SA, Porto, Portugal

INTRODUCTION

The study of HMW (high molecular weight) compounds in sugar refineries is important for technical and product quality reasons. Godshall et. al (1998, 1992) studied HMW compounds separated by GPC using a refractometric index detector and ultraviolet detection. In this study samples were concentrated through dialyses.

The use of a Light Scattering Detector associated to a diode array detector (DAD) was presented recently (Bento et al, 1997, Bento and Sá, 1997). With this system the study of HMW compounds not detectable even by UV spectrophotometry can be made. Due to the high sensitivity of this detector it is not necessary to concentrate samples or separate HMW compounds by dialyses or other means. In the previous work it was observed that HMW compounds were separated by the Superose column into three groups. Group A comprised compounds removed from the column at R_t (retention time) between 20 and 31 minutes, corresponding to compounds with MW higher than 250 kD (column calibrated with dextran). This group comprises compounds with low colour, possibly associated with polysaccharides. In affination, compounds of this group remain preferentially in the affined sugar crystals.

The second group, Group B, with R_t between 40 and 48 minutes, corresponded to compounds with MW between 10 and 43 kD. These compounds have a high colour intensity.

The third group, Group C, corresponds to compounds with R_t between 48 and 58 minutes with MW from 2.5 kD to 10 kD.

Compounds with MW lower than 2.5 kD are not considered in this study as they are not separated from sugars and other low MW compounds.

It was observed in these previous works that compounds with higher molecular weights have a tendency to remain in sugar crystals (are crystallophilics) and lower MW compounds are rejected to syrups and are present in majority in molasses (60%).

In this paper the same technique is used to study HMW compounds after affination (affination liquor), carbonatation (carbonatation liquor) and resin decolourisation (fine liquor).

A DAD spectrophotometric detector is placed in series with an evaporative light scattering detector (ELS) in order to evaluate colourant types and colour intensities.

LIGHT SCATTERING DETECTOR

The ELS is a sensitive detector for non volatile solutes in a volatile liquid stream. The eluent enters the detector at the top of the evaporation chamber. (Figure 1). The eluent/solute is fed into the nebulizer assembly where a venturi jet operated by air atomises the eluent into a uniform dispersion of droplets, which then pass as a continuous stream into the evaporator. When pure eluent is being evaporated, only its vapour passes through the light path and the amount of light scattered to the photodetector is small and gives a constant response. When non-volatile solute is present, a particle cloud passes through the light, causing light to be scattered. This scattered light enters the optical aperture of the detection system and generates a signal response from the photomultiplier. The signal is amplified and gives a voltage output. The quantity of light detected is dependent on the solute concentration and solute particle size distribution (PL-EMD960 Operator's Manual).

Values of ELS detector in figures are in μ Volts. When only effluent is passing in detector base line response is 10000 μ Volts.

MATERIALS AND METHODS

Equipment

Pharmacia FPLC system equipped with a liquid Chromatography Controller LLC-500 Plus and High Precision Pump P-500.

Column: Pharmacia, 300 mm length and 10 mm internal diameter, packed with Superose 12, cross-linked, agarose-based medium, with an average particle size of 10-11 μ m, and an exclusion limit of circa 2×10^6 g/mol (globular proteins).

Evaporative light scattering detector (ELS) PL-EMD 960 Polymer Laboratories.

Lichograph Diode Array Detection System (DAD) L-4500 Merck-Hitachi, continuous absorption measurement in the UV/VIS range.

Ultra-sounds: Bandelin, Sonorex TK30.

Membranes Nylaflo, Gelman Sciences

Reagents

Acetonitrile gradient grade for chromatography -MERCK;

Ammonium acetate - MERCK;

Polyethylenglycol 35000 - MERCK.

Eluent and sample preparation

An exigency of the use of ELS detector is to use an eluent with a low boiling point to be evaporated inside the detector. In this study we used distilled water with acetonitrile, 30%, containing 0.005M ammonium acetate. The eluent, at pH 7.0, was filtered through a 0.45 μm membrane and then degassed 15 minutes with ultrasound.

Samples, 20 g, were diluted in eluent up to 50 ml and adjusted to pH 7.0. Solutions were filtered through a 0.45 μm membrane and degassed for 5 minutes with ultrasound.

For comparison each liquor was diluted to the same Brix in order to have the same amount of dry substance in each injection.

Because samples were filtered through 0.45 μm in order to protect the chromatographic column, very high molecular weight compounds are removed and are not considered in this study.

Synthetic colourants were prepared as described in the literature (Shore *et al.*, 1984).

Run method

Before each run, the system was stabilised for a period of 30 min by passing eluent at a flow rate of 0.30 ml/min. Run time was 59 min and 200 μl sample was injected. With this running time sucrose does not enter the ELS detector.

Conditions at the ELS detector were: airflow at 5 l/min and air temperature 80°C. For security reasons a flow of nitrogen was fed continuously into the detector case.

Analytical methods

Phenolics were determined using Folin and Ciocalteu reagent and dextran using the AOAC Roberts method.

RESULTS AND DISCUSSION

Samples were taken in the refinery allowing for residence time at each stage. Analytical results are presented in Table 1. ELS chromatograms are presented in Figures 2 to 6.

Quantification of groups of HMW compounds

In order to quantify the compounds in each group, sample was mixed with PEG (polyethyleneglycol) at a concentration of 0.2 g/100 ml giving 0.04 mg at each sample injection. PEG is separated in the Superose column with a retention time of 34 minutes, between Group A and B.

Comparing areas of the PEG peak with areas of the three groups we can quantify these compounds in ppm equivalent to PEG (Figure 7). Results are presented in Table 2.

Identification of groups of HMW compounds

In order to try to identify the colourants present in each Group, by the ELS detector are presented in Figures 13, 14 and 15. Results are presented in Table 3.

Discussion

It is observed that compounds of Group A are not greatly removed during carbonatation and resin decolourisation.

Carbonatation

Compounds of Group B are 80% removed during carbonation, indicating that these compounds must have a strong anionic nature. This group of compounds is associated with melanoidins.

Compounds of Group C increase globally during carbonatation. An increase of 51% of these compounds were observed in the sample studied.

It is known that reducing sugars are destroyed at high alkalinity forming hexoses alkaline degradation products (HADP) compounds. When affined liquors are mixed with calcium hydroxide pH increases above 10 and at this high alkalinity reducing sugars can be destroyed and colourants formed. A destruction of 80% of reducing sugars was observed. The increase of peak C1 indicates the formation of HADP. With the technique used here we can observe the colour formation during carbonatation. By global colour measurement this is not possible. As this group of colourants is one of the major groups present in white sugar (Figure 8) it is important to study the carbonatation conditions in order to minimise this colour formation.

During carbonatation peak C2 is reduced. This peak does not present great colour intensity.

Decolourisation

Decolourisation at RAR is done in two stages of 6m³ resin columns. The same polystyrenic divinyl benzene strong base anionic resin is charged in each column.

Compounds of Group B are almost completely removed during this step. A reduction of 88% was observed, confirming the anionic character of these compounds. The presence of melanoidin compounds in this group confirms the affinity of these compounds for anionic resins (Guimarães et al., 1996).

Group C compounds remain almost the same after resin treatment although peak C1 decreased and peak C2 increased in the sample in this study. The fine liquor sample was taken from one resin system in the beginning of the liquor cycle with 9 BV of feed liquor decolourisation.

To study the increase of peak C2 during resin decolourisation, a test was made by collecting samples during a resin cycle. A pilot plant from Applexion with 80 litres of resin was used. A resin cycle of 25 hours at 2BV/hour of carbonated liquor with 441 IU colour was performed. Decolourisation of 92% was obtained.

Samples of fine liquor and corresponding carbonation liquor were taken during the resin cycle. Results of variation of Group C compounds during the resin cycle are presented at Figures 9, 10, 11 and 12.

It was observed that peak C1 is much more removed in the beginning of the working cycle and this removal decreases with cycle length.

Referring to Peak C2 it is observed that in the beginning of the cycle a higher peak is observed in relation to the incoming liquor. During the resin cycle this peak decreases attaining a minimum at the end.

Analysing the average sample of carbonated and fine liquor it is observed that peak C1 decreases and peak C2 is maintained after resin decolourisation.

As it was observed in DAD analysis this peak presents low colour intensity.

One possibility to explain this increase of C1 peak, higher than that of the incoming carbonated liquor, is the presence of amphiphilic compounds that can switch from hydrophobic to ionic bond fixation mechanisms during resin wash at low salt concentration (Bento, 1992). In the next cycle these compounds are dislodged by others with more affinity to resins. In a previous work (Bento, 1992) it was proved that these compounds are dislodged from the resin by an acid wash following the normal regeneration with NaCl.

It was observed that some inorganic anions are excluded earlier from the Superose column. Retention times of some anions are: phosphate - 51.76 min; sulphate - 50.72 min; chloride - 51.00 min. Acetate and carbonate ions are excluded after 60 minutes. Sodium and calcium cations are also excluded after 60 minutes. The presence of chloride in fine liquor when a new resin is used is observed in the beginning of

the decolourisation cycle. In Figure 16 the peak corresponding to chloride appears between peaks C1 and C2. This confirms the previous hypothesis.

CONCLUSION

The study of HMW compounds with GPC using a Light Scattering detector is useful to study the behaviour of different compounds during carbonatation and ion exchange resins.

When a process presents a global decrease of colour it is difficult to detect any colour formation. With the technique used in this study it is possible to evaluate the colour formation during carbonatation and the behaviour of different compounds during resin decolourisation.

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The authors wish to thank their RAR/DID colleagues, Applexion for providing the resin pilot plant and the financial support from PRAXIS Program to the RECICLAMAS Project.

Table 1. Liquors analysis.

	Affination Liquor	Carbonatation Liquor	Fine Liquor
Brix	67.9	67.5	66.6
Colour IU	1325	583	278
IV	2.30	2.78	2.91
pH	7.8	8.3	8.6
Phenols mg/l	182	107	71
Dextran ppm	414	395	406

Table 2. Quantification of HMW compounds referred to PEG*.

	Group A	Group B	Group C
Affination Liquor	229 ppm	490 ppm	576 ppm
Carbonatation Liquor	211 ppm	99 ppm	870 ppm
Fine Liquor	212 ppm	---	896 ppm

* ppm on dry matter of sample.

Table 3. Results with synthetic colourants.

	Rt (ELS) min	MW kD	Group
Caramel	51.88	5.5	C2
Melanoidin	44.12	21.1	B
"	51.66	5.7	C2
HADP	50.64	6.9	C1

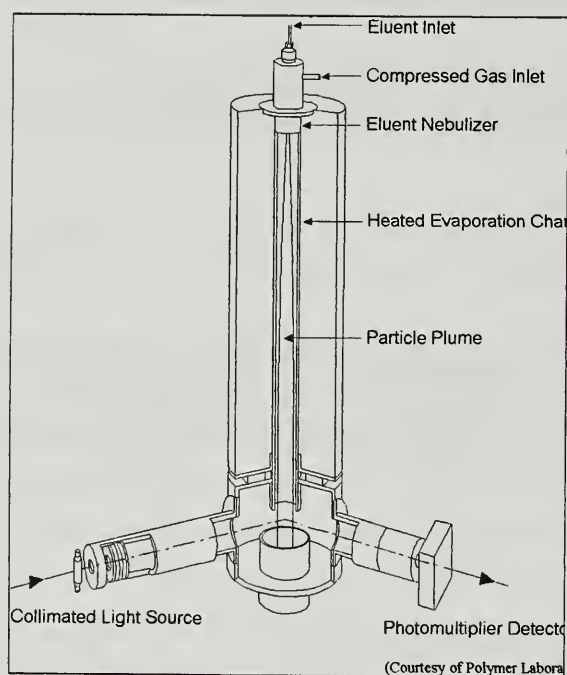


Figure 1. ELS detector.

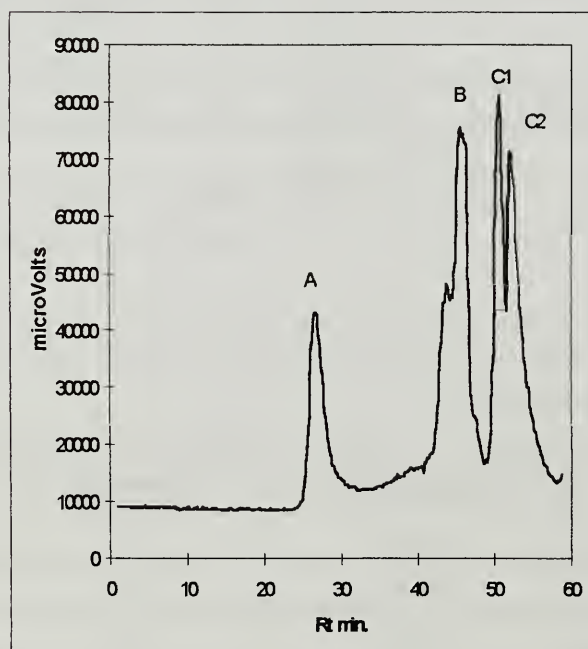


Figure 2. Affination Liquor.

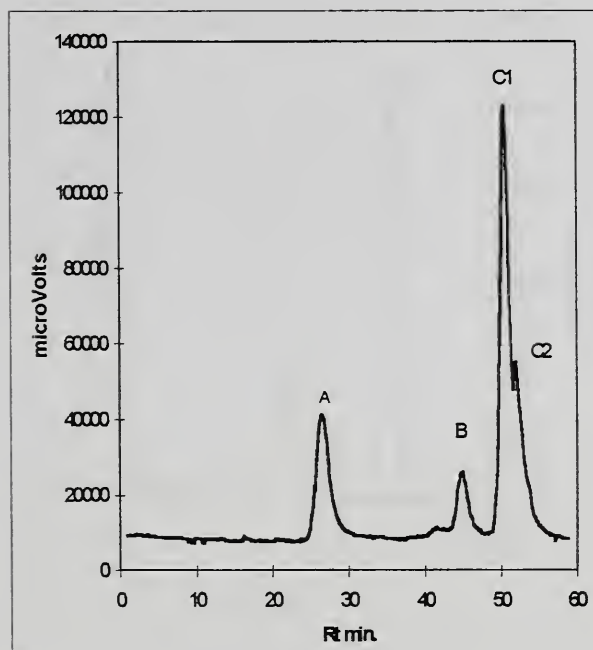


Figure 3. Carbonatation liquor.

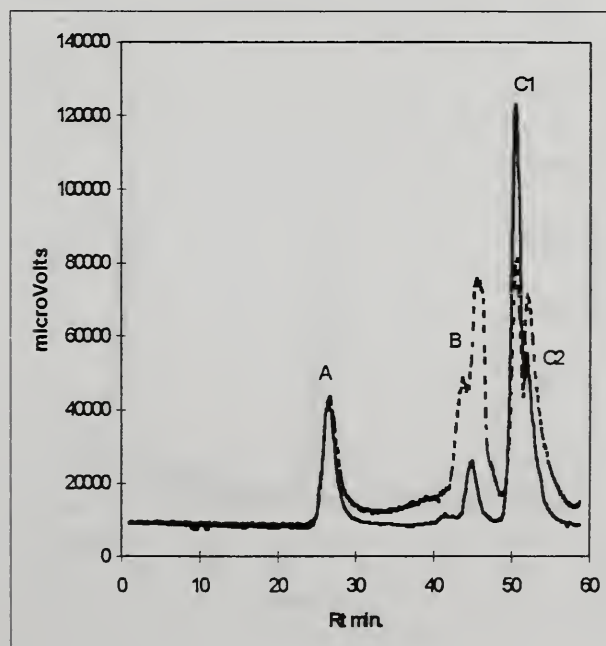


Figure 4. Affination (---) and carbonatation liquor.

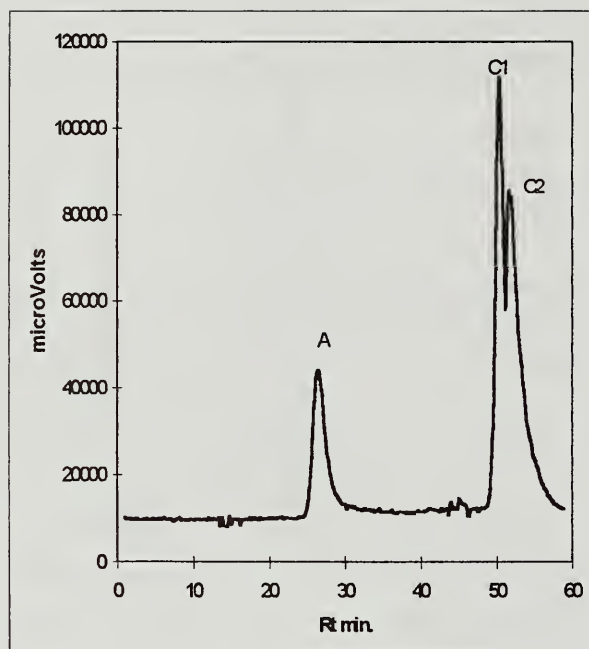


Figure 5. Fine liquor.

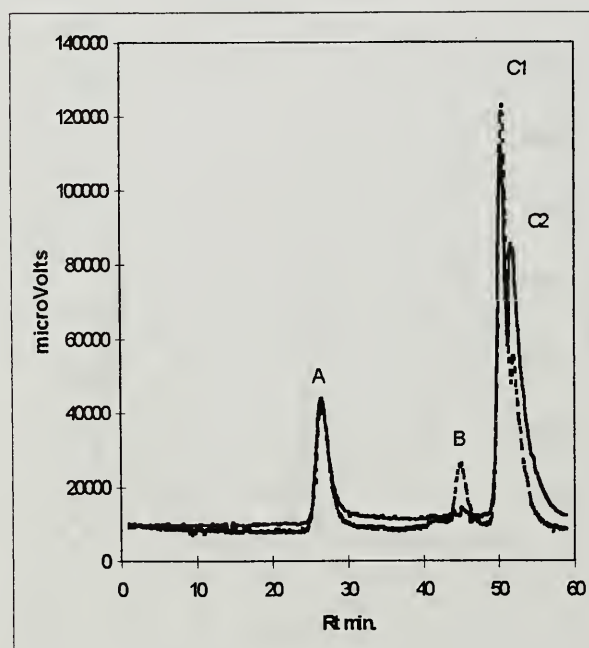


Figure 6. Carbonatation (---) and fine liquor.

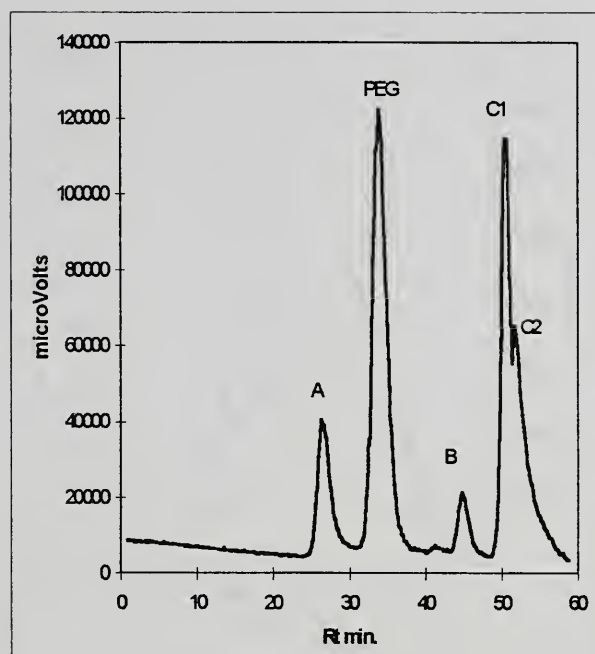


Figure 7. Carbonated liquor + PEG.

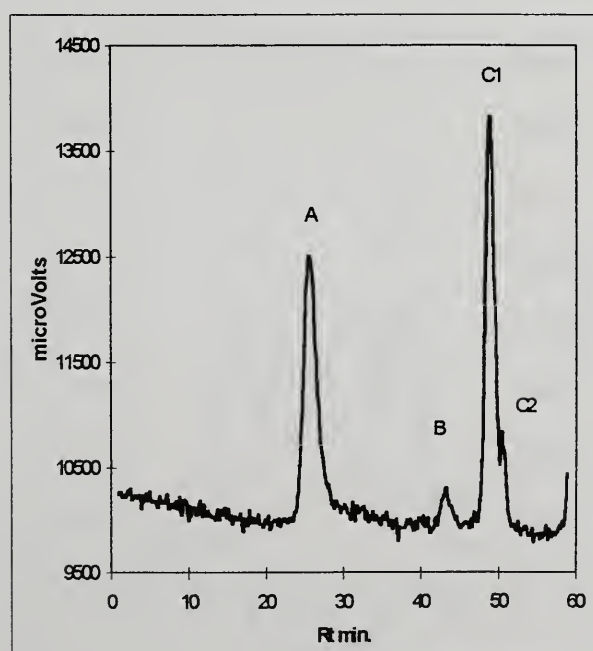


Figure 8. White sugar.

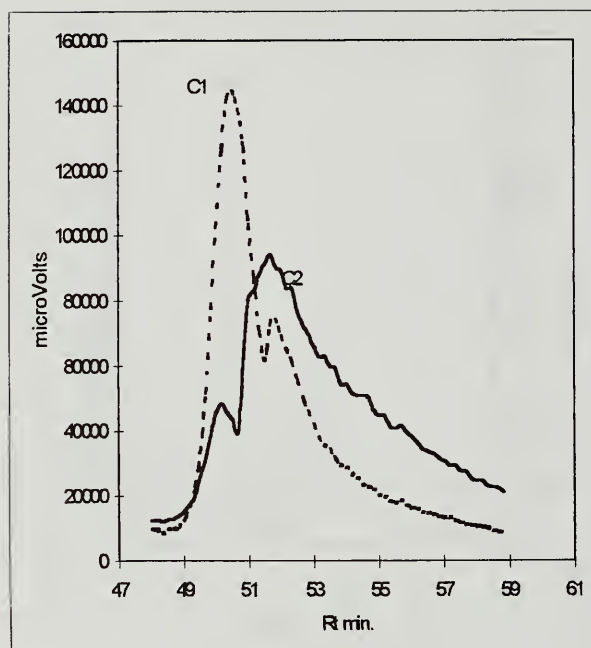


Figure 9. Resin cycle: beginning (carbonatation (---) and fine liquor).

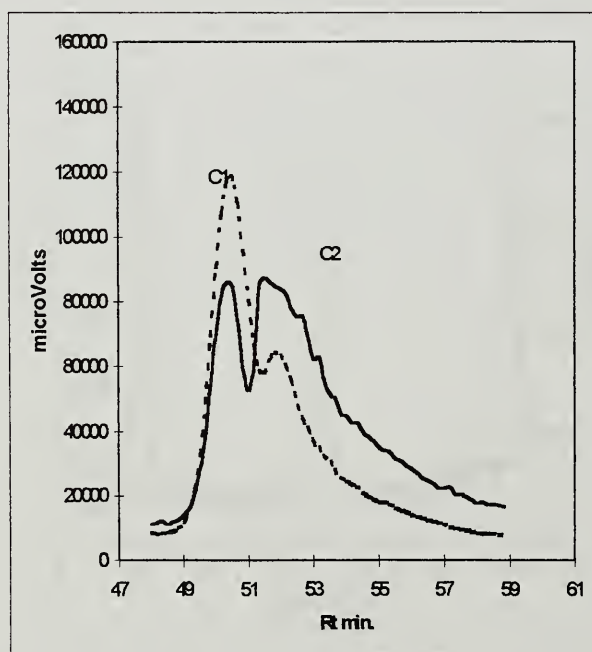


Figure 10. Resin cycle: middle (carbonatation (---) and fine liquor).

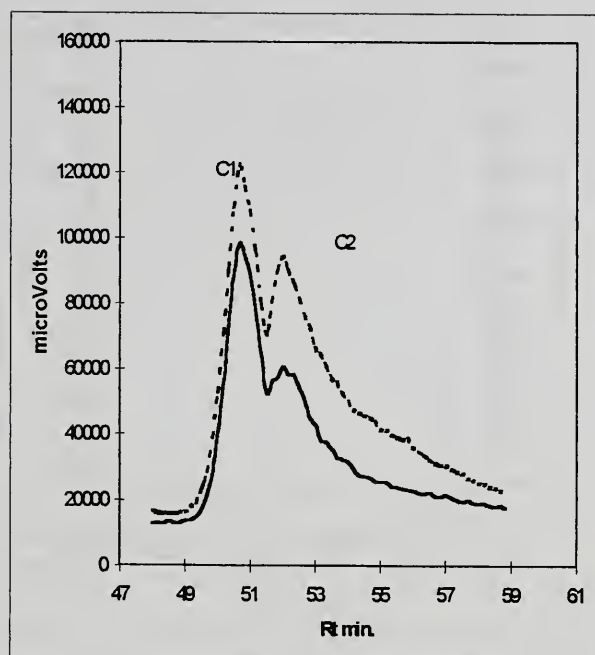


Figure 11. Resin cycle: end (carbonatation (---) and fine liquor).

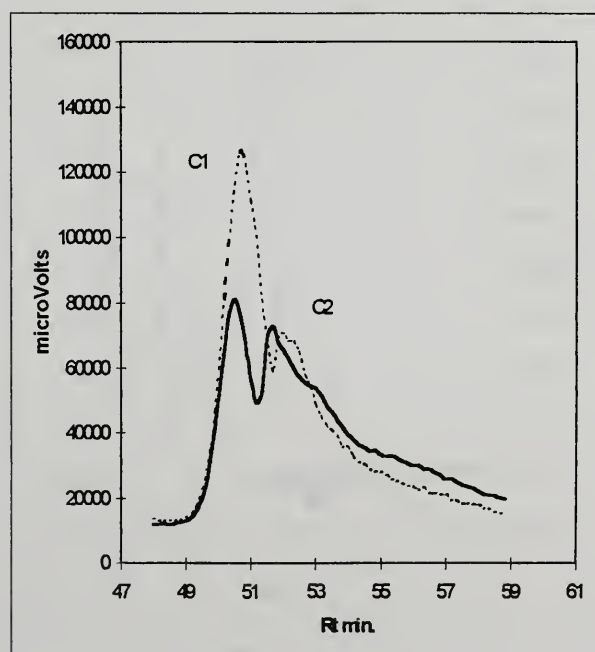


Figure 12. Resin cycle: global (carbonatation (---) and fine liquor).

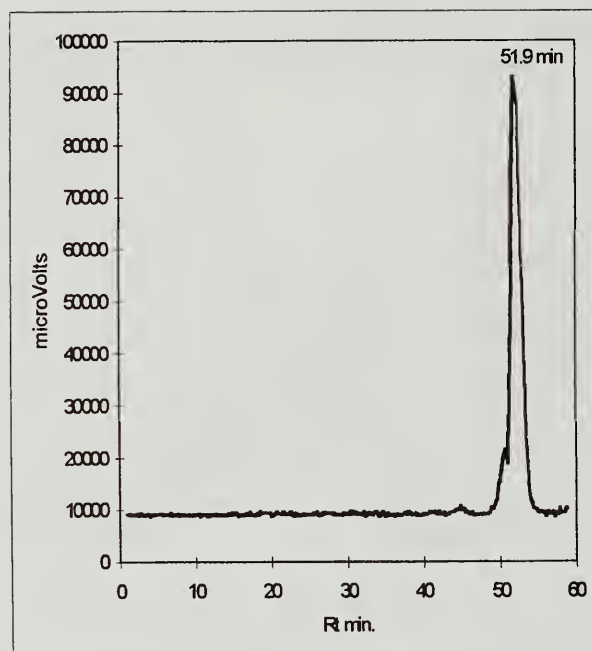


Figure 13. Caramel.

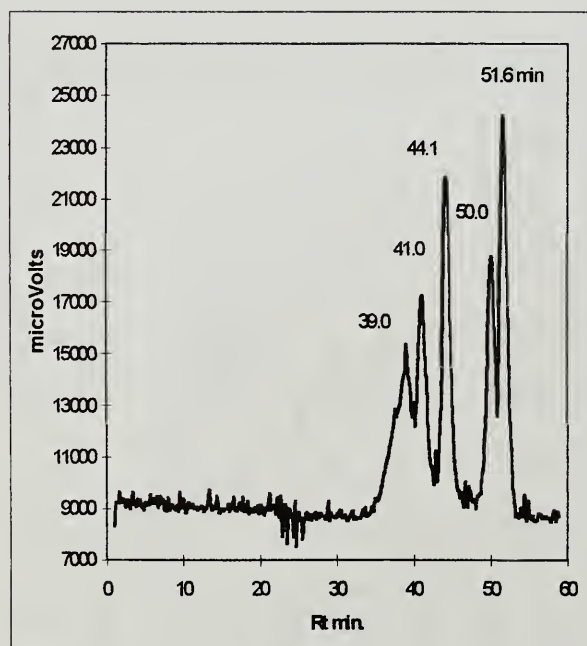


Figure 14. Melanoidin.

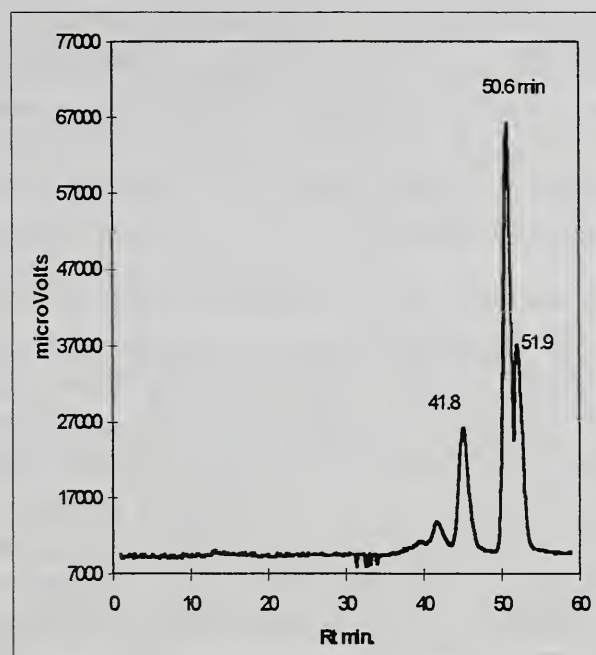


Figure 15. Hexoses alkaline degradation products (HADP).

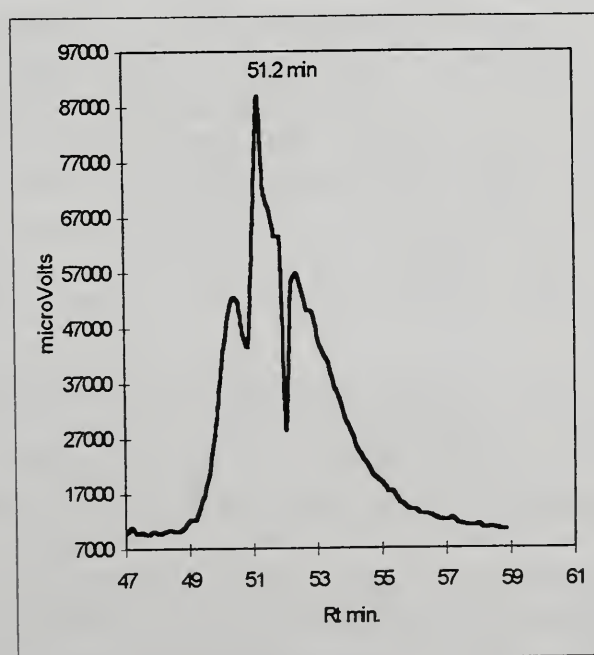


Figure 16. Fine liquor (new resin) (beginning of cycle).

MEMBRANE SEPARATION CHEMISTRY IN SUGAR PROCESSING APPLICATIONS

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ABSTRACT

Membrane technology in the beet and cane sugar industries is being investigated to improve clarification of juices, to partition colorant prior to evaporation and crystallization, and to improve exhaustion of molasses for increased sugar recovery. The Sugar Processing Research Institute, Inc. (S.P.R.I.) has compared synthetic organic membrane systems as well as ceramic cross flow, colloidal, and ultrafilters to effect these processes. Chemists of S.P.R.I. have defined raw material such as crusher juice or molasses fractions entering the membrane streams as well as each of the fractions coming from the membrane retentates and permeates. Subfractionation of the material by membrane nominal molecular weight cutoff types afforded enriched high polymer and permeates of lower molecular weight colorant or components which were further analyzed by gel permeation chromatography, compositional analysis, and physical methods such as nuclear magnetic resonance spectroscopy. Colloidal chemistry of molasses retentates indicates that affinity of the natural components produces very high molecular weight aggregates which are implicated in membrane fouling and decrease in flux and throughput.

INTRODUCTION

Feasibility of using membranes in the sugarcane and sugarbeet process industries was established early in the development of industrial membranes (12,22) to separate sucrose from colorant and other molecules that interfere with crystallization. Bichsel (3,4) described purification of dilute molasses solutions with nylon membranes that recycled permeate to provide constant feed composition. Although this work (4) was in the initial stages of commercially available membranes for chemical process applications, Bichsel set the stage for much future development of membranes in all of the sugar industry. Madsen (40,41,42), at Danish Sugar, contemporary with Bichsel's work, developed reverse osmosis and ultrafiltration equipment that was applicable on a large scale to processing in the sugar industry.

Developments in separations of sugar juice and molasses fractions by membrane systems has led to a whole new technology. Kishihara (29) reported elimination of color in affination syrup by ultrafiltration and decolorization of the ultrafiltrates with adsorbents. The same author (30,31) advanced membrane technology in the field of sugar processing by reporting clarification of technical sugar solutions through a dynamic membrane formed on a porous ceramic tube, and then applied the technology to define the effect of factory sugar solution on growth of sucrose crystals. Hanssens and coworkers (23) described in 1984 the promise of ultrafiltration as an alternative for raw juice purification in the beet sugar industry. Chang used ultrafiltration in 1985 as a method of concentrating high molecular weight compounds in raw sugar for the analysis and characterization of some cane sugar impurities (10).

As membrane technology expanded in the sugar industry, applications such as those proposed by Wilson and Percival in 1990 (60) emerged wherein ultrafiltration was proposed as a new alternative for the management of regenerant waste streams. Saska and De Lataillard (49) reported in 1993 the concentration and decolorization of dilute products from cane molasses desugarization with reverse osmosis and nanofiltration membranes. From this development Saska and coworkers (50) in cooperation with the Applexion group (36,37) then proposed in 1995 the direct production of white cane sugar with clarification and decolorization membranes. The development of tangential microfiltration (cross flow membranes) brought much greater efficiency to the applications to sugar processing since colloids in the sugar juice were less likely to foul the membranes (12,18).

A series of papers have described the use of ceramic colloidal and ultrafilters to sugar processing problems from the research of the Rhone Poulenc research laboratories. These membranes are composed of a hard porous baked alumina/titania support on which is bonded zirconium oxide to effect membrane pore size or selectivity. For example, the Kerasep membrane is marketed by TechSep and used in the engineering applications of Applexion. In particular, the names of Lancrenon, Theoleyre and Cartier (6,7,8,9,36,37) from the Applexion development group have applied these ceramic membranes to production of refined sugar at the cane sugar mill, treatment of affination syrup by micro/ultrafiltration and its impact on the crystallization process, and the use of mineral membrane filtration for the corn refining industry. Kwok, Lancrenon, and Theoleyre announced in 1996 a patented process of manufacturing crystal sugar from an aqueous sugar juice such as cane juice or sugar beet juice using the ceramic membranes Applexion has engineered with the original Rhone Poulenc product (35). Kwok has carried these studies forward on the factory scale (33,34) with ultrafiltration/softening of clarified juice as a door to direct refining and molasses desugarization in the cane sugar industry and in several other papers defined ultrafiltration/softening of clarified sugarcane juice using the new Applexion process.

In a separate set of developments, using other membranes, Monclin presented in 1996 the "A.B.C. Process" for the direct production of refined sugar from cane mixed juice (44). This was based on Monclin's patents in 1995 (45,46) which describe a process for producing refined sugar directly from sugarcane and a separate process for producing refined sugar from raw sugar. In both of these patents the sugar refining is effected by: "A", clarification of the raw juice or raw sugar solution by extensive screening; "B", ultracentrifugation or ultrafiltration to polish the juice or syrup to high translucence; and "C", removal of certain compounds responsible for adverse color quality and viscosity through a set of packed columns filled with an absorbent for these compounds. After evaporation and crystallization, refined cane sugar is produced, thus the trade name "A.B.C Process". Juszczak (27) has done considerable work at NIRO Engineering on membrane filtration applications in the sweetener industries, following the lead of workers such as Madsen from Der Danske Sukkerfabrikker. In a recent review (12), the Danish membrane work is summarized for cane sugar membrane applications in sugar mills, refineries, and for syrups. In addition, applications to clarification of beet and cane juice is described.

As a whole other topic, Juszczak (27) also extends his discussion to the use of these same membranes in the starch-based sweetener industry for dextrose and high fructose corn syrups. The rapid growth of membrane technology in the sugar industries is illustrated in the recent paper by Kochergin (32) on membrane filtration of various sugar solutions in beet sugar production. In addition, Kochergin and

others, at this 1998 Sugar Processing Research Conference, have brought up to date all of the above cited literature used to put into perspective this present paper.

In the present study, high concentrations of suspended colloidal solids, polysaccharides, browning polymers, plant cell wall debris, and other unknown materials in sugar processing streams which block membrane systems by slowing flux and altering membrane pore properties are studied. As contrasted to the studies above which targeted properties of industrial membranes used for sugar recovery and their physical performance to create a new industrial approach to sugar production, emphasis is being given here to the use of these same industrial membranes for employment in preparative separations of low and high molecular weight components. Cogent problems addressed in this paper pertaining to the development of membrane processes for successfully improved efficiencies in both sugarcane and sugarbeet sectors are the following:

- Use of preparative membrane techniques to quantitate, characterize, and define unknown compositional factors in processing juice or syrup which coalesce to form evaporator buildup or impede sugar crystallization during molasses exhaustion.
- Isolation and characterization of indigenous sugar juice components which drastically change membrane flux rates, with intent to remediate fouling problems by chemical, biochemical, or physical processes not yet currently applied.
- Early inhibition of component reactivity or degradation, and diversion of troublesome fractions into separate sugar recovery streams to permit economically efficient primary process streams with high yields of sucrose.

Preparative membrane chemistry and gel chromatography can be used to both isolate and break apart the tight network of sugarcane and sugarbeet polymers that to the present defy technology intended to improve sugar recovery. The various molecular weight ranges of substrates for polymer formation as well as the products generated under the stress of juice evaporation were examined using membrane processes found effective in sugar processing. In previous papers by the Sugar Processing Research Institute, coworkers have reported (5,14,15,16,17,20,21,58) chromatographic separations at various stages of the postharvest sugar purification process and across the harvest season to characterize fractions and delineate polymeric by-products. The present work extends membrane separation and polymer characterization to investigate also sugarbeet syrups and molasses.

EXPERIMENTAL - METHODS AND MATERIALS

Sugarcane evaporator syrup, "A" molasses, "B" molasses, and final "C" molasses were taken as individual samples (not a daily composite at the process source) for analyses described below from a Louisiana sugar factory at appropriate locations in the process stream during the 1995 harvest (58). Samples of sugarbeet ion exclusion separator fractions were taken on two separate days in the 1996 processing period and labeled "Feed molasses", "Extract", and "Raffinate" (32).

Analytical methods were taken from the International Commission for Uniform Methods of Sugar Analysis *Methods Book*, April, 1994 (25) and Methods of the Association of Official Analytical Chemists, 16th edition, Ch. 44, pp. 44-1 through 44-43 (17) as well as S.P.R.I. methods.

Analyses performed included pol, refractometer Brix, apparent purity, pH, color, dextran, haze (26), polysaccharides, starch and dry substance solids by heated vacuum oven drying. High performance liquid chromatography of sucrose, glucose, and fructose for the Louisiana factory samples and sugarbeet ion exclusion separator samples was performed on a Dionex PAC-1 column with pulsed amperometric detection using the Dionex AI-450 integrating computer program (1). The column was run at ambient temperature with 100 mM carbonate free sodium hydroxide in purified water (18 megohm-cm resistivity) and flow rate of 1.0 ml/min with a 200 mM sodium hydroxide wash at the end of the run and re-equilibration to 100 mM eluent before the next run. Samples were filtered through a 0.45 μ M nylon filter prior to injection. Calibration responses with 2-amino-2-deoxy-glucose internal standard (57) were run repeatedly over the entire period of sampling and membrane separation experiments.

Gel permeation chromatography for colorant polymers was carried out according to Khan and coworkers (28) as well as Saska and Oubrahim (48) using a Shimadzu high performance liquid chromatography system equipped with an SPD-6AV ultraviolet detector and RID-6A refractive index detector using the Shimadzu LC-10AD pump, an SPD-6AV ultraviolet-visible detector, an RID-6A refractive index detector, and Rheodyne 7125 injection port. A 100 μ l injection sample loop was employed for samples filtered through 0.2 μ l filter prior to injection. Three TSK-GEL PW-XL columns in order as G6000, G5000, and G4000, corresponding to decreasing pore sizes, with appropriate TSK guard column. Samples were uniformly diluted to 15° Brix with elution solvent prior to filtration. Solvent was 0.1 M sodium chloride in purified water (18 megohm-cm resistivity) that was modified with 10 % HPLC grade acetonitrile. Flow rate was 0.6 ml/min. The ultraviolet detector was set at 210 nm. Calibration standards were purchased from Scientific Polymer Products, Inc., New York, NY. These standards are sodium polystyrene sulfonates of molecular ranges from 4000 Da to 1.2 million Da. A Dionex AI-450 laboratory data analysis system was used to integrate peaks as well as to sample peak area slices for polymeric calibration curves and molecular weight determinations.

Application of membrane ultrafiltration to soluble molasses polymer isolation was carried out as in Vercellotti et al. (57) with the following organic membrane modules. Two Cordis Dow C-DAK, cupraphan regenerated cellulose, preparative hollow fiber dialyzers (1500 Da nominal molecular weight cutoff (NMWCO) (each 1.8 m²) were staged with a Cole-Parmer Masterflex peristaltic pump to deliver 2 liters/min through the fibers with circulating tap water through housing to carry off permeate for desalting higher molecular weight retentates. Colloidal filtration was effected on a Fresenius polypropylene P2S Plasmaflux cross flow hollow fiber plasma filter with 0.2 μ M average pore size and 2×10^6 Da NMWCO with 2 to 5 l/min flow rate through the fibers and flux of 0.5 l/min of permeate collected from outside the fibers through the housing. Collected permeate or diafiltrate was concentrated on a Dow Filmtec BW30-2514 spiral wound, reverse osmosis nanofilter with pore size 0.001-0.005 μ M (Minneapolis, Minnesota, USA) using a rotary vane pump (Procon, Murfreesboro, Tennessee, USA) at 100 psig (689 kPa) which achieves permeate at 1 l/min (concentration rate of 40 liters to 1-2 liters in 3 hours depending upon osmotic pressure) (12,57). Ceramic membrane modules were obtained from Applexion, 78681 Epone Cedex, France (Kerasesp, Rhone Poulenc; 1 m by 2 cm; 0.45 μ M, 0.2 μ M, 0.1

μM , 300,00 Da, and 15,000 Da NMWCO pore size)(6,7,8,9,36,37). Solutions were pumped with a Procon rotary vane pump at 120 psig (820 kPa) at ambient or 50° C. Permeates or retentates were concentrated on the reverse osmosis unit described above. Prior to membrane filtration the molasses samples (about 1 kg at 80° Brix) were diluted to about 15° Brix and centrifuged on an International PR7000M programmable centrifuge with a 6 x 1 liter, #966 head capacity. Centrifugation was carried out at 5000 rpm in balanced, screw top 1 liter polypropylene bottles (Nalgene) for 30 minutes at 8°C. Supernatants were siphoned off with an aspirator to avoid particulate in the membrane system, and combined for colloidal filtration. Pellets of insoluble molasses debris were dried in a vacuum oven for compositional analysis.

Preparative gel filtration was carried out on high molecular weight colloidal filter retentates of sugarcane Final C molasses and raffinose from sugarbeet molasses ion exclusion separation. Approximately 2 liters of Sephadex G-200 (Pharmacia), swollen in 0.08 M potassium phosphate buffer, pH 6, was allowed to settle in an 85 x 300 mm column equipped with a sintered glass frit covered with fine glass wool and stopcock. The column was calibrated with blue dextran (Pharmacia) and potassium dichromate. The column was allowed to equilibrate by gravity with the same buffer over a day and about 5 grams of retentate loaded on and washed slowly into the column with buffer with a flow rate of 2 ml/min. Fractions of 10 ml were collected on a Buchler fraction collector for a total solvent flow of 3110 ml. Fractions were pooled after reading at 280nm in the ultraviolet. Fractions were concentrated by rotary evaporator and characterized. Nuclear magnetic resonance spectroscopy of Sephadex G-200 fractions was effected on a Bruker n.m.r. spectrometer with 90 MHz carbon and 360 MHz proton fields using samples saturated in perdeuterated methylsulfoxide (DMSO-d₆). Gas chromatography was effected on extracts or hydrolyzates of the extracts as trimethylsilyloximes of sugars or trimethylsilyl esters or ethers of other functional groups (38) using a DB-5 capillary column (30 m; 0.53 mm i.d. coated with 0.33 μM phenyldimethylsiloxane). Sugars were estimated in the hydrolyzates of fractions as alditol acetates on the gas chromatograph with an SP 2340 column (Supelco, Bellefonte, PA, USA) according to Theander (51,52) and Englyst (19).

RESULTS AND DISCUSSION

Sugarcane Molasses

The objective of this work has been to carry out preparative organic chemical separations on final C molasses using industrial membranes to isolate suitable quantities of sugarcane or sugarbeet polymers that are interactive in membrane processing steps. Careful characterization of the colloidal material involved in membrane separations of sugar processing streams, understood in terms of structural organic chemistry, will lead to remediation measures which will permit economically feasible operations in the sugar factories (11). Membrane fractionation of sugarcane final C molasses is shown on Figure 1 using organic polymer colloidal filters. Membranes employed are described in the EXPERIMENTAL section above. The distribution of higher molecular weight material rejected at various membrane porosities represents a size distribution of material interfering with juice or syrup clarification. In this preparative molasses polymer isolation work, the Sugar Processing Research Institute, Inc., has used both organic and ceramic membranes successfully. From many years of experience we know that in such a preparative

chemistry application it is essential, for both membrane types, to clarify the molasses samples by centrifugation at 7000 or more times gravity as a relative centrifugal force (on our 6 liter, preparative laboratory centrifuge that is about 5000 rpm) to avoid changing properties of the colloidal filter or ultrafilter or foul the membrane so that preparative flow rates are not useful. The nature of colloidal material being put forward past dissolved solid materials, filters, or clarifiers, and being concentrated in sugarcane final C molasses as heat induced polymers or coordination networks with ash, is such that it is not surprising that some 9.3% of the molasses is sedimentable on initial centrifugation. The nature of this pelleted material and related substances is being considered.

The cross flow colloidal filtration on the 0.2 μ M polypropylene hollow fiber unit goes smoothly at room temperature. About 2 hours are required to complete filtration of the 8 liters of molasses supernatant. We continuously dialyze with the two 1500 Da cutoff dialyzers against tap water circulating around the dialyzer housing while maintaining a concentration level of about 6 liters by adding water when the osmotic pressure no longer permits replacement of water by diffusion. We monitor the dialyzate as well as the feed using a conductivity meter. By the time the retentate is completely filtered, the conductivity levels off to almost that of tap water. If the dialyzate is kept for preparative purposes, it is concentrated with a reverse osmosis unit. The retentate is likewise concentrated with the reverse osmosis unit and then finally concentrated for freeze drying or alcohol precipitation on a rotary evaporator. The viscous retentate is highly colored and is almost black. The permeate of the colloidal filter can be ultrafiltered again or simply dialyzed with the same system as described above. The results confirm other reports of yields of polymeric, non-dialyzable material in the final C molasses (10,16,17,20,21,49,53,54,55). Each of the isolated fractions in Figure 1 was then characterized. For example, in Figure 2 the high molecular weight fractions in the retentate (>1 million Da) are shown to be well separated from those of the permeate on ultrafiltration on analytical gel permeation chromatography. The development of the very high molecular weight compounds, >1 million Da polymers found in final C molasses, is traced from evaporator syrup through the B molasses in Figures 3 and 4. We have repeated such experiments with gel permeation chromatography of fractions of sugarcane molasses from membranes many times and these high molecular weight peaks are universal, no matter where the sample originates.

To discern the nature of the colloidal retentate of sugarcane final C molasses, the dialyzed retentate was subjected to size exclusion chromatography on the cross-linked dextran, Sephadex G-200 (15,16). The exclusion limit on Sephadex G-200 is about 1 million Da, with an effective fractionation range of 5000 to 800,000 Da. As described in the EXPERIMENTAL the retentate was separated into 6 fractions as shown in Figure 5. The column diagram gives an idea of the distribution of molecular weights across the elution volume from Blue Dextran marker (2 million Da, completely excluded) to potassium dichromate (gram formula weight 294, totally included). The column calibration was essential to characterize the nature of this colloidal molasses aggregate.

In Table 1 the distribution of total polysaccharides, dextran, and starch in gel filtration fractions of colloidal retentate from sugarcane final C molasses is presented. It was a great surprise to find that in such a highly pigmented mixture Fraction 1 is nearly colorless. In fact, pigmentation and color bodies actually increase going to lower molecular weight (left to right) (2). In Table 1, the polysaccharide composition of Fractions 1 through 6 are shown and include significant quantities of polysaccharide, dextran, and starch. By far the greatest dextran concentration is in Fraction 1 although it is spread

throughout the fractions in lesser quantity. The fact that various molecular weight distributions of all these polymers is present demonstrates the heterogeneous nature of the plant source from which they come. The distribution correlates with earlier observations (10,16,17,20,21,49,53,54,55) that high molecular weight colorants are selectively occluded in the crystals during crystallization.

In Table 2 the carbohydrate composition of the polysaccharides from these gel filtration fractions is shown. The carbohydrates are normalized to glucose as 100%. Fraction 1 is highest in glucose although mannose is also present possibly as an end-group component of polysaccharide from wild-type bacteria producing dextran (47). The simplest relationships among the monosaccharide containing fractions would indicate molecular weight distributions of dextran, arabinogalactan, arabinoxylan, soluble xylan, and various cell wall debris which also contains beta-1-3 glucan. The mannose content of Fraction 6 is higher than in other fractions. Fraction 6 is very low molecular weight (<1000 Da) and the origin of these components could be degraded glycoprotein or contaminating yeast mannan. Degree of hydrolysis drops off going to the lower molecular weight materials indicating that the polysaccharide character decreases and other kinds of polymer such as polyphenols predominate (13,20,21). The colloidal nature of these fractions from the colloidal retentate is demonstrated in Table 3 where haze analysis is compared for three polymeric mixtures. The membrane retentate has the highest haze quantity.

Analytical gel permeation chromatography was performed on each of the Sephadex G-200 fractions using Toyo Soda TSK columns as described in the EXPERIMENTAL (2,28,48,58). In Figure 6 the very high molecular weight distribution of Fraction 1 is compared with Fraction 2, which loses much of the polymer near the void volume. Both ultraviolet and refractive index detection were used to detect the relative masses injected on the column and to characterize the aromatic content of each (2,39,59). As can be seen comparing Fractions 1 and 2, Fraction 1 contains about a third of the intensity of ultraviolet absorbing material of Fraction 2. Similarly, in Fractions 3 and 4 (Figure 7) the intensity of ultraviolet absorbing material is higher than in Fractions 1 or 2. The greatest absorbance is seen in the lowest molecular weight fractions, Fractions 5 and 6, which are coincidentally very highly colored.

The proton nuclear magnetic resonance spectra of sugarcane final C molasses colloidal retentate fractions from gel filtration demonstrate some of the same observations made in the previous paragraph about their analytical gel chromatography (28). In Figure 9, the protons of anomeric carbons in the region 5.2-5.4 ppm are indicative of an alpha-D-linked polysaccharide and probably can be attributed to dextran's backbone of alpha-1-6 glucan (28). Other pyranose ring protons in the 3 to 5 ppm chemical shift range are also typical of such a structure. No obvious aromatic resonances are found in the proton spectrum of Fraction 1, giving the impression that it is predominantly polysaccharide as concluded above.

Fraction 2 begins to have aromatic resonances in the chemical shift range of 6 to 9 ppm. In Figure 10, Fractions 3 and 4 continue to have polysaccharide character in proton clustering but they indicate much more of the lower energy resonance of more highly substituted aromatic rings, probably polymers from the peak broadening. Fractions 4 and 5 (Figure 11) have high intensities of these broad aromatic peaks in the 6 to 10 ppm range. The somewhat lower intensity of aromatic rings and greater resolution of proton chemical shifts at higher energy (aliphatic) would indicate that Fraction 6 is predominantly low molecular weight material of a mixed carbohydrate and substituted aromatic nature. Although more complex than the present treatment requires, it should be mentioned that ^{13}C nuclear magnetic resonance

spectra of these retentate gel filtration fractions confirm the polysaccharide and aromatic nature of the proton assignments above.

From all of the above it is clear that a strong aggregate forms of high and low molecular weight material in the colloidal filtration of sugarcane final C molasses. Each of the fractions examined were involved in actual processes that fouled membranes. Why these polymers fill the narrow pore size of the membranes even when the viscosity is relatively low and flow rate is quite rapid is not understood. These are transverse flow membranes (cross flow at a 90° angle to the flux) and generally can resist fouling (12). Trapped in this aggregate, held together with electrostatic and other hydrophobic or hydrophilic forces, are high molecular weight dextrans, polysaccharides, and polyaromatics which, in turn, build up complex aggregated layers on the membrane surface and eventually cause fouling and diminished flux. Much more work must be done on other insoluble residues carried along in the colloidal filtrate. An unexpected aspect of this treatment was that in the retentate, even after long dialysis, sucrose was still entrapped in the aggregate. A comparison of the integral role of sucrose in membrane concentration of molasses and its inclusion in the retentate aggregate will be given below.

Sugarbeet Molasses

As the further application of similar membrane deterioration phenomena resulting from components of molasses, colloidal and ultrafiltration were applied to processing streams involved in sugarbeet ion exclusion separators for enhanced sugar recovery. Many such separator applications are now in operation in beet sugar technology (32). In general, separation of feed molasses in sucrose recovery processes results in a sucrose enriched extract and a higher molecular weight ion exclusion fraction termed the raffinate. Comparison of the three components in the ion exclusion separator gives an appreciation of the efficiency of the operation. Thus, in Table 4, 80% ethanol fractionation (43) of sugarbeet ion exclusion feed molasses, extract, and raffinate indicated that the feed molasses contains much more soluble material than the raffinate (81% vs 58%) with the corresponding ethanol insoluble material accumulating in the raffinate (24% vs 41%, respectively). The extract, however, is completely soluble in ethanol. The ethanol extract solutions were stored in the refrigerator for more than six months before crystals began to appear. It was concluded that the center cut extract from the ion exclusion column contained much low molecular weight material which prevented sucrose crystallization, perhaps acting as an emulsifier or solubilizing agent. The solids from these alcoholic partitionings are in themselves interesting and contain much sucrose entrapped in the polymeric network. There may be specific compounds in this mixture that act as crystallization initiating agents.

ICUMSA colors of the ion exclusion separator fractions described in the previous paragraph are shown in Table 5 (13,14,15,16,25). The high color value of the raffinate at all pH's is indicative of the concentration ability of the ion exclusion separator to segregate and concentrate colorant away from the sucrose-rich isolate in the extract. That is not to say that the extract is already colorless, but that compared to the quality of feed molasses, the extract is a considerable upgrade in quality. In effect, the raffinate probably contains more easily removed colorant while the more difficult to remove remains in the extract. Sugar concentration in these same ion exclusion separator samples is shown in Table 6. The

concentration gradient to the extract fraction is well emphasized in the purity of the raffinate (17%) compared to the extract (77%).

During the course of studying the sugarbeet ion exclusion separator fractions for their chemistry in membrane processing, it became clear that interesting polymers would probably be concentrated in the highly colored raffinate. We did membrane separation processes, similar to separations described above for sugarcane final C molasses, on the sugarbeet ion exclusion feed molasses, extract, and raffinate, with careful examination of all of the components isolated. In the present report is described separation of the ion exclusion raffinate in Figure 12. This separation was carried out on the ceramic Kerasep membranes described above in the INTRODUCTION and EXPERIMENTAL sections as an illustration that the same chemical separations can be carried out on either membrane type (36,37). As done above, the success of colloidal filtration could only be guaranteed after centrifugation at 7200 x gravity for 0.5 hour to pellet suspended particulates somewhat larger than the 0.2 μ M pore size of the filter.

The sugarbeet raffinate contained much less insoluble solids in the pellet than the sugarcane final C molasses but substantial enough to dry and weigh (4.6 g or 0.7% of Brix solids in Figure 12).

During the course of colloidal filtration through the 0.2 μ M pore size ceramic membrane, continuous dialysis was used to reduce ionic strength and lower osmotic pressure as above. The sugarbeet ion exclusion raffinate had much better flux through the membrane than the sugarcane final C molasses described earlier. It can only be concluded that the sugarbeet syrups and molasses are more efficiently filtered and/or softened prior to ion exclusion separation probably because of their low content of polysaccharides. However, much brown-black color remained in the retentate even though not as much high polymer was present. In the subsequent steps of ultrafiltration through 300,000 Da and 15,000 Da nominal pore size membranes intense color persisted through the permeates, but a large residue of color remained in the retentates (6,7,8,9).

Because of interest in characterization of the polymers in the raffinate colloidal filter retentate, about 5 g of that material was placed on a Sephadex G-200 column as above. The experimental conditions and fractionation effected are shown on Figure 13. In the case of the raffinate retentate much less complicated polymer structure was present. Although not fully characterized as yet, these six gel filtration fractions have very similar properties to those from the sugarcane final C molasses retentate above. Fraction 1 is apparently mostly only lightly colored polysaccharide. This is probably indigenous sugarbeet polysaccharide.

Colorant increased as the retention time increases in the column so that the most highly colored fractions are those with the lowest molecular weights. Figure 14 shows the analytical gel permeation chromatogram of the sugarbeet ion exclusion feed molasses and the extract. There is little or no high molecular weight material in either of these chromatograms, which would have only been expected for the extract and not for the feed molasses. Similarly in Figure 15 the raffinate contains more high molecular weight material than the feed and the greatest amount of ultraviolet absorbance of all. As an indication of the position of the exclusion volume on the elution scale of Figure 15, a 1.2 million Da standard is run for comparison. The ultraviolet data for the areas under the curves in Figures 14 and 15 actually reflect ratios similar to those ICUMSA absorbances in Table 5.

Membrane Fouling Complex

Assuming, therefore, that the retentate of the sugarbeet ion exclusion raffinate on colloidal filtration is also an aggregate that can clog membranes and cost considerably more energy to pump through the ultrafilters, there probably also is sucrose being entrained in the polymers.

Table 7 shows the distribution of residual sugars in concentrated retentates and permeates of sugarcane final C molasses and sugarbeet ion exclusion extract after treatment with a 15,000 Da Kerasep ceramic membrane with extensive dialysis with the hollow fiber 1500 Da cutoff dialyzers. Both of the feed molasses are expected to have high sucrose content. The permeate of the final C sugarcane molasses harbors much sucrose (7.2%) even after extensive dialysis indicating that the polymeric material in the dialyzed retentate, bracketed between 1500 to 15,000 Da, is preventing the sucrose from being stripped out by the dialyzer. The higher molecular weight retentate above 15,000 Da also resists dialysis of sucrose and holds 6.4% residual sucrose. The same experiment with sugarbeet extract indicates that the native constituents of the original feed molasses hold sucrose to an even greater extent than did the sugarcane polymers. Thus, the dialyzed permeate bracketed between 1500 and 15,000 Da held some 55% sucrose, and the corresponding dialyzed retentate, with molecular weight greater than 15,000 Da, held 26.2%. Interaction of these sugarbeet molasses polymers with the Kerasep ceramic membrane could be altering the surface in a way that the constituents of the sugarcane final C molasses do not. The question of efficiency of membranes under potentially surface modifying conditions is open, and one which will be an interesting experiment to pursue.

SUMMARY AND CONCLUSIONS

Interactions of sugarcane and sugarbeet juice or processing syrup components during membrane filtration with synthetic organic membrane systems, as well as with ceramic crossflow colloidal filters and ultrafilters, result in complex membrane surface modifications. The major factors involved in solute separation by membranes are 1) intrinsic rejection by the membrane of a specific solute, and 2) the nature of the concentration polarization layer at the surface of the porous filtration medium.

Factors involved in determining the behavior of membranes in sugar processing have been defined for raw material such as crusher juice or molasses fractions entering the membrane streams as well as each of the fractions coming from the membrane retentates and permeates. Subfractionation of the material by membrane nominal molecular weight cutoff types afforded enriched high polymer and permeates of lower molecular weight colorant or components which were further analyzed by gel permeation chromatography, compositional analysis, and physical methods such as nuclear magnetic resonance spectroscopy.

Colloidal chemistry of molasses retentates indicates that affinity of the natural components produces very high molecular weight aggregates, which are implicated in membrane fouling or other inefficiencies of the process. Intrinsic rejection of solutes by the membranes and the dynamics of concentration polarization of the membrane surface were found to be very important physical properties in the feasibility of using membrane processes to reduce energy input, maintenance costs, and effect improved

yields of purified sugar. Reduction of tightly held, colloiddally suspended particulates in sugar juices or syrups by physical means before membrane filtration is extremely important to ensure the success of reducing the concentration of color bodies or crystallization inhibitors.

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Table 1. Distribution of total polysaccharides, dextran, and starch in gel filtration fractions of colloidal retentate from sugarcane final C molasses.

Percentage of residues based on lyophilized dry weights			
Sample name	Total polysaccharide (%)	Dextran (%)	Starch (%)
Fraction 1	23.00	9.49	0.20
Fraction 2	5.16	1.65	0.25
Fraction 3	17.73	0.61	0.20
Fraction 4	2.04	0.59	0.18
Fraction 5	34.73	0.81	0.16
Fraction 6	8.18	1.61	0.16

Table 2. Carbohydrate composition of sugarcane final C molasses retentate fractions from separation on preparative gel filtration column.

Alditol acetates by gas chromatography on hydrolyzates after dialysis						
	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
	Percent sugars	Percent sugars	Percent sugars	Percent sugars	Percent sugars	Percent sugars
Rhamnitol	0.36	0.56	0.00	2.31	2.75	3.27
Arabinitol	0.83	18.60	20.95	19.72	20.07	41.92
Xylitol	0.60	5.12	12.85	7.81	11.47	20.45
Mannitol	3.66	3.02	0.89	4.18	5.28	12.68
Galactitol	0.33	17.32	5.71	9.45	11.47	5.73
Glucitol	73.68	55.38	45.99	43.77	48.97	15.95
Total recovery of sugars on hydrolysis (%)	91.57	89.30	67.75	71.27	44.56	47.41

Table 3. Haze analysis of typical ultrafiltration samples and fractions from sugarcane final C molasses.

Sample	°Brix	Dextran mg/kg on solids
Sugarcane molasses (feed) Final C	16.9	1112
Retentate of mw 15000 cutoff Final C molasses (DIALYZED)	58.7	7532
Permeate of mw 15000 cutoff Final C molasses (DIALYZED)	75.7	292

Table 4. Ethanol fractionation of sugar beet ion exclusion feed molasses, extract, and raffinate.

Molasses 6-27 75° Brix		Extract 6-17 66° Brix		Raffinate 6-17 67° Brix	
Wet weight	239.7 g	Wet weight	306 g	Wet weight	310.7 g
Dried pellet	44.9 g	Dried pellet	None	Dried pellet	85.39 g
Pellet, wet	18.73%	Pellet, wet	None	Pellet, wet	27.48%
Pellet, dry	24.97%	Pellet, dry	0.00%	Pellet, dry	41.01%
Soluble in ethanol	194.8 g	Soluble in ethanol	306 g	Soluble in ethanol	225 g
Soluble	81.27%	Soluble	100.00%	Soluble	58.99%

Table 5. ICUMSA color of sugarbeet molasses extracts.

		Absorbance at 420 nm			ICUMSA color units			Absorbance ratio of ICU's at pH9/ICU's at pH4
Sample	pH as rec'vd	pH 4.0	pH 7.0	pH 9	pH 4.0	pH 7.0	pH 9	
Beet molasses 6/27/96	8.3	0.298	0.538	0.638	49667	89667	106333	2.14
Extract beet molasses	9.3	0.052	0.068	0.082	8667	11333	13667	1.58
Raffinate beet molasses	6.7	0.491	0.972	1.128	81833	162000	188000	2.30

Samples were diluted 1 to 10 to give Brix initially 1/10 that of the molasses, and then again diluted 1 to 5 for reading on the spectrophotometer. The dilution of the original molasses is given in Brix.

Table 6. Sugar concentration in sugarbeet molasses ion exclusion samples.

Sample 1 June 17, 1996	Feed molasses	Sugar fraction extract	Raffinate
°Brix	75	66	67
Apparent purity	54	77.5	17.1
Sucrose % on sample	40.5	51.15	11.5
Glucose % on sample	6.5	4.2	10.1
Fructose % on sample	0.04	0	4.2
Sample 2 Nov. 18, 1996	Feed molasses	Sugar fraction extract	Raffinate
°Brix	77	68	67
Apparent purity	47.26	82.38	25
Sucrose % on sample	36.39	56.02	16.75
Glucose % on sample	9.19	1.51	1.36
Fructose % on sample	1.29	0.09	0

Table 7. Residual sugar determination in membrane concentration fractions.

	Percent sugar in syrup on μ G basis				
	Glucose	Fructose	Sucrose	Unknown	Unknown
Sugarcane final C molasses feedstock Not ultrafiltered or dialyzed	8.12	6.68	42.26		
Sugarcane final C molasses - Applexion permeate from 15,000 Da cutoff Kerasep ceramic membrane. Extensively dialyzed with 1500 Da cutoff hollow fiber membranes	0	0	7.16		
Sugarcane final C molasses - Applexion retentate from 15,000 Da cutoff Kerasep ceramic membrane. Extensively dialyzed with 1500 Da cutoff hollow fiber membranes	0.86	0.79	6.39	0.06	
Sugargeet ion exchange "extract" Not ultrafiltered or dialyzed	0.06	0.15	50.45	4.43	0.12
Sugarbeet ion exclusion "extract" - permeate from Applexion 15,000 Da cutoff Kerasep ceramic membrane. Extensively dialyzed with 1500 Da cutoff hollow fiber membranes	0.04	0.07	55.63	0.75	0.3
Sugarbeet ion exclusion "extract" - retentate from Applexion 15,000 Da cutoff Kerasep ceramic membrane. Extensively dialyzed with 1500 Da cutoff hollow fiber membranes	0	0	26.23		

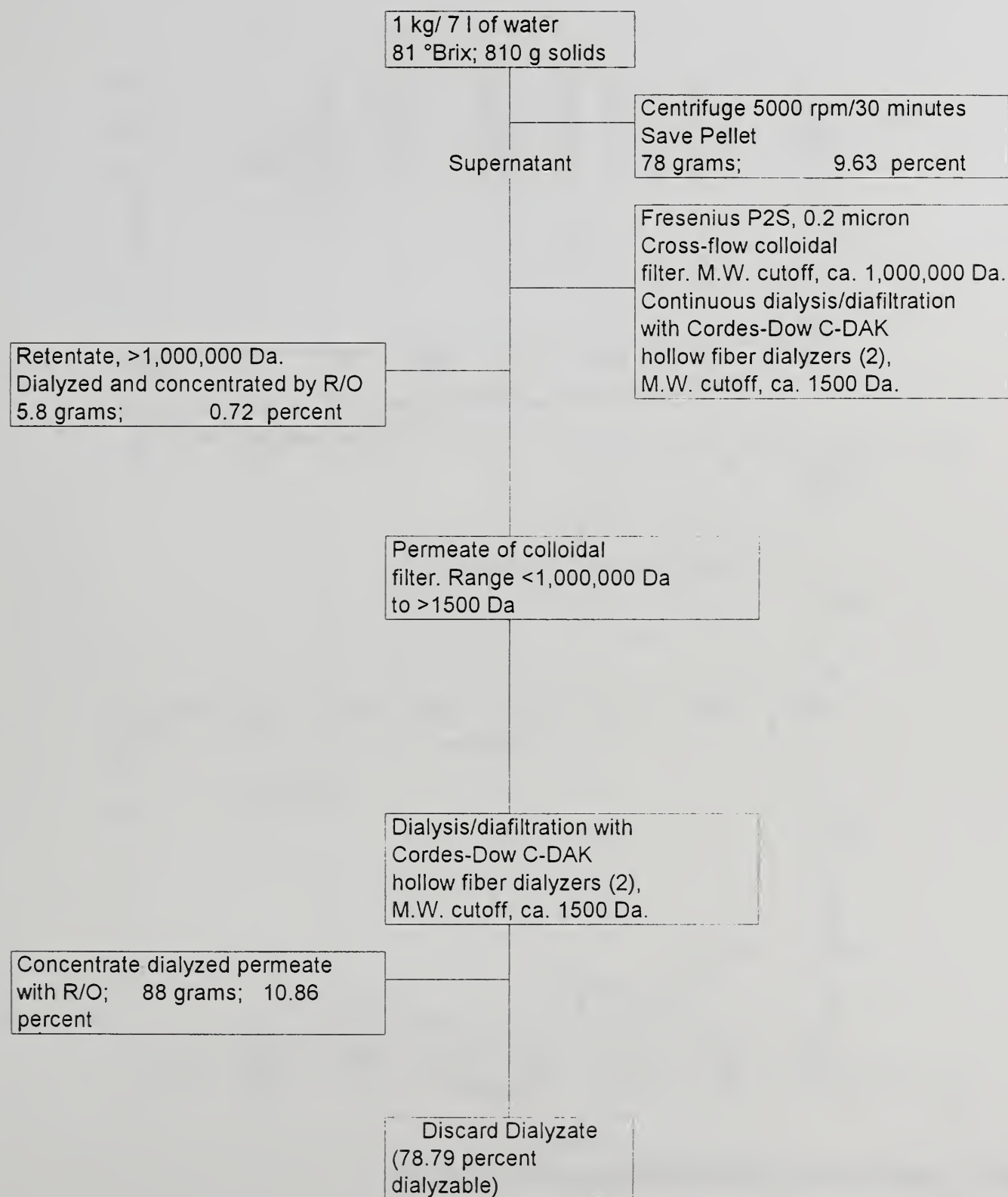


Figure 1. Ultrafiltration of sugarcane final C molasses on organic polymer colloidal filters.

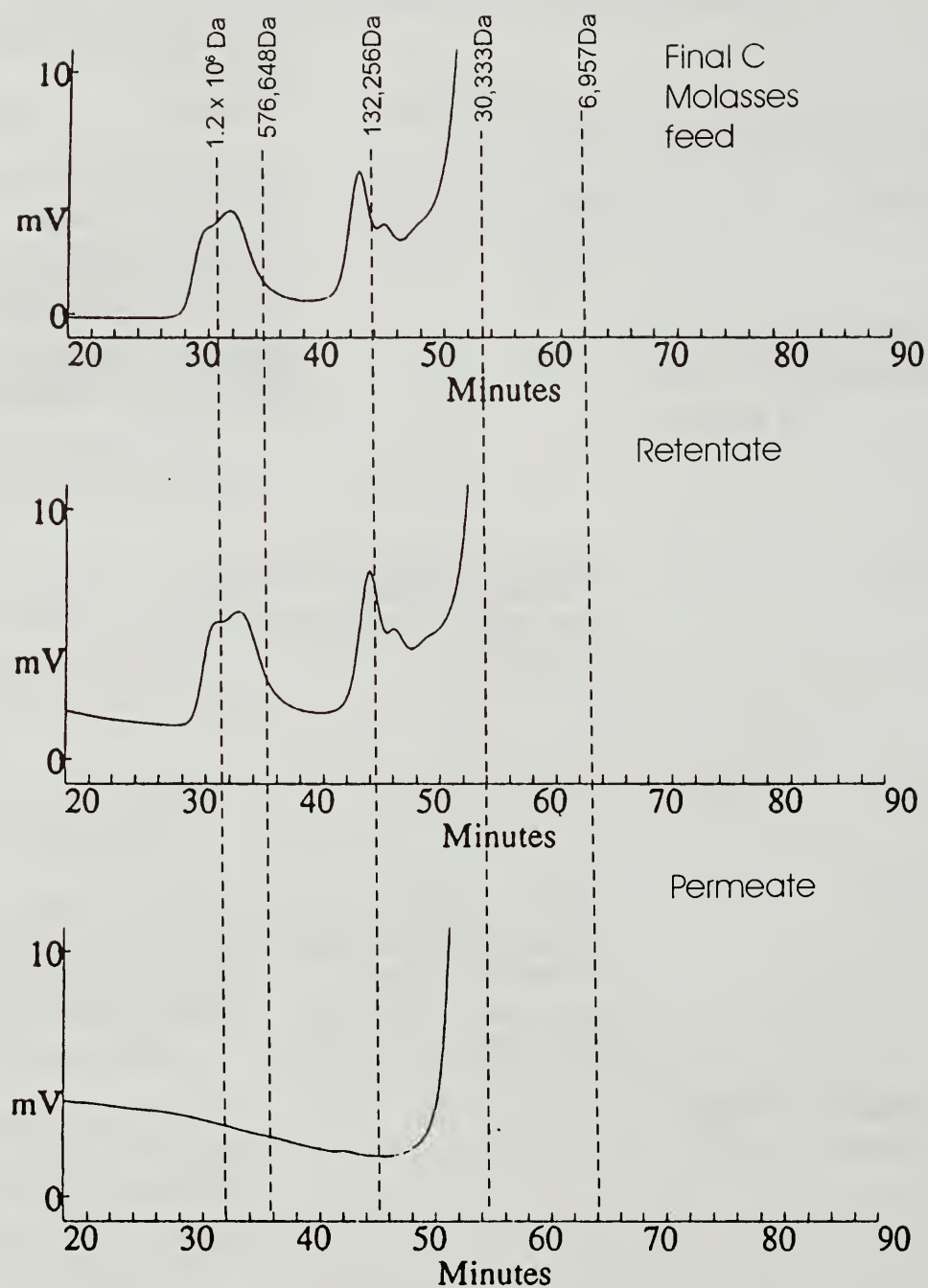
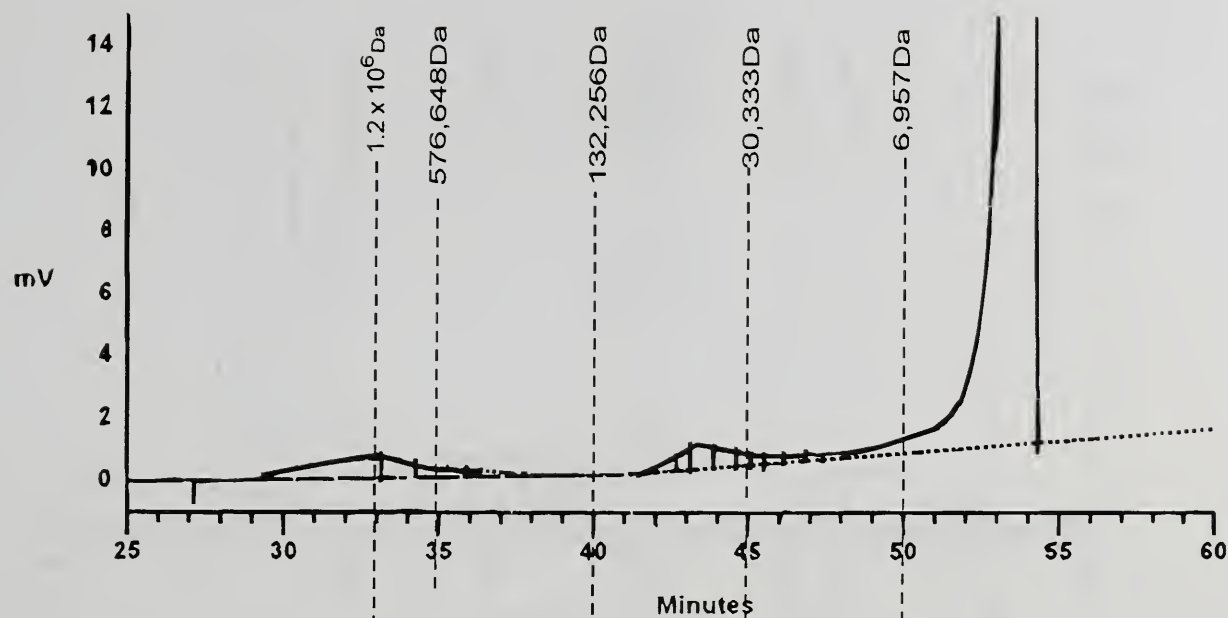
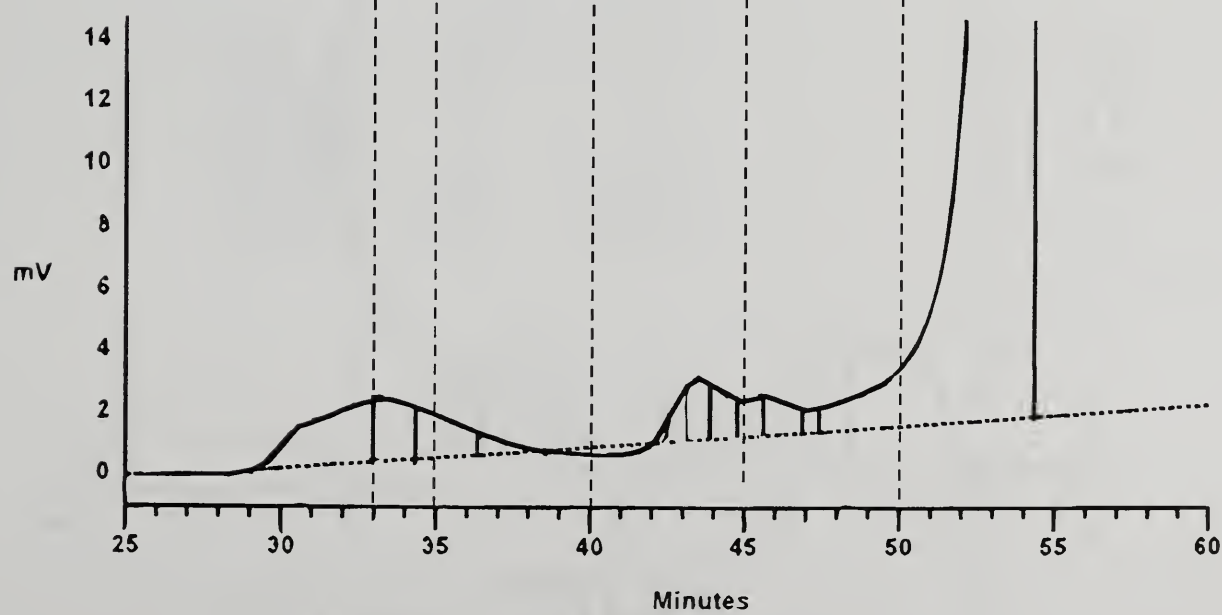


Figure 2. Applexion filtration of final C molasses UV detector.

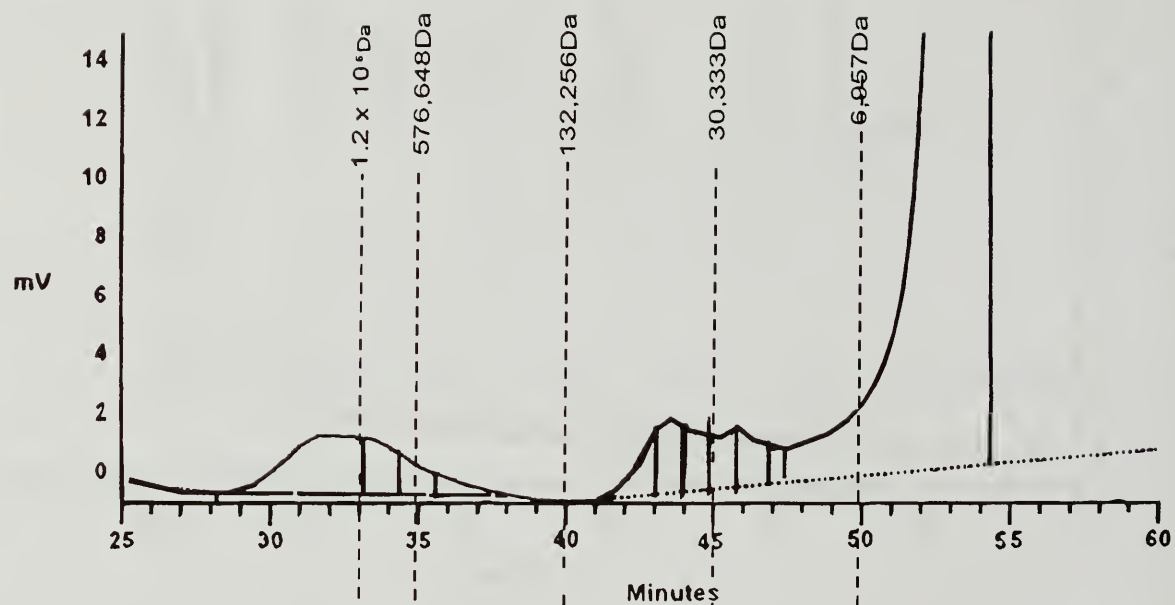


Evaporator syrup 10-27

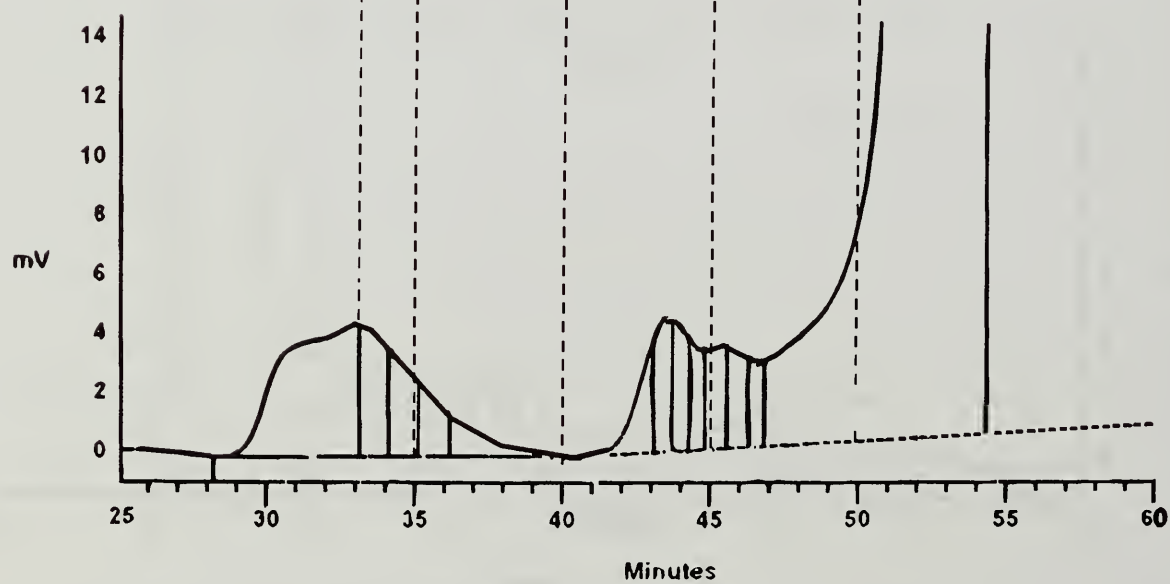


A Molasses 10-27

Figure 3. Evaporator Syrup 10-27 and molasses A 10-27.



B Molasses 10-27



Final C Molasses 10-27

Figure 4. Molasses B 10-27 and final C molasses 10-27.

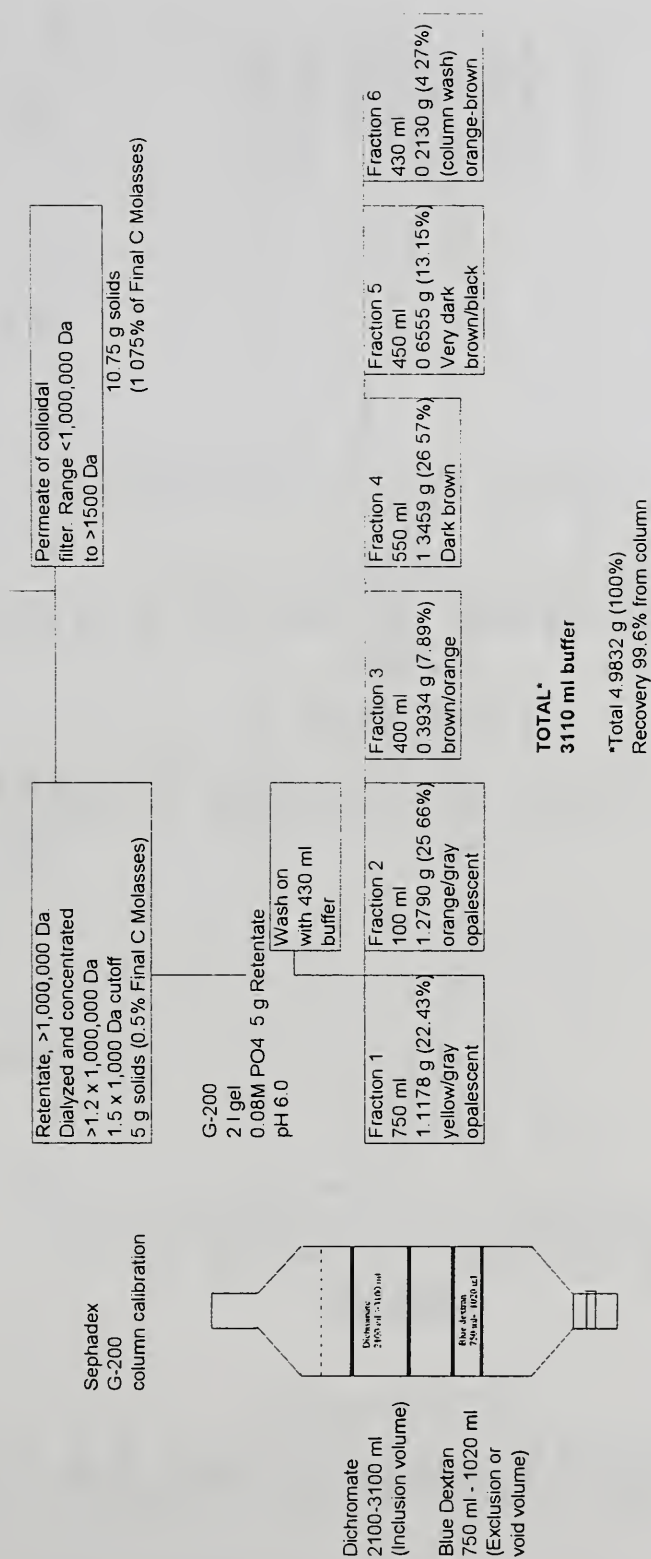


Figure 5. Preparative gel filtration of sugarcane final C molasses colloidal retentate.

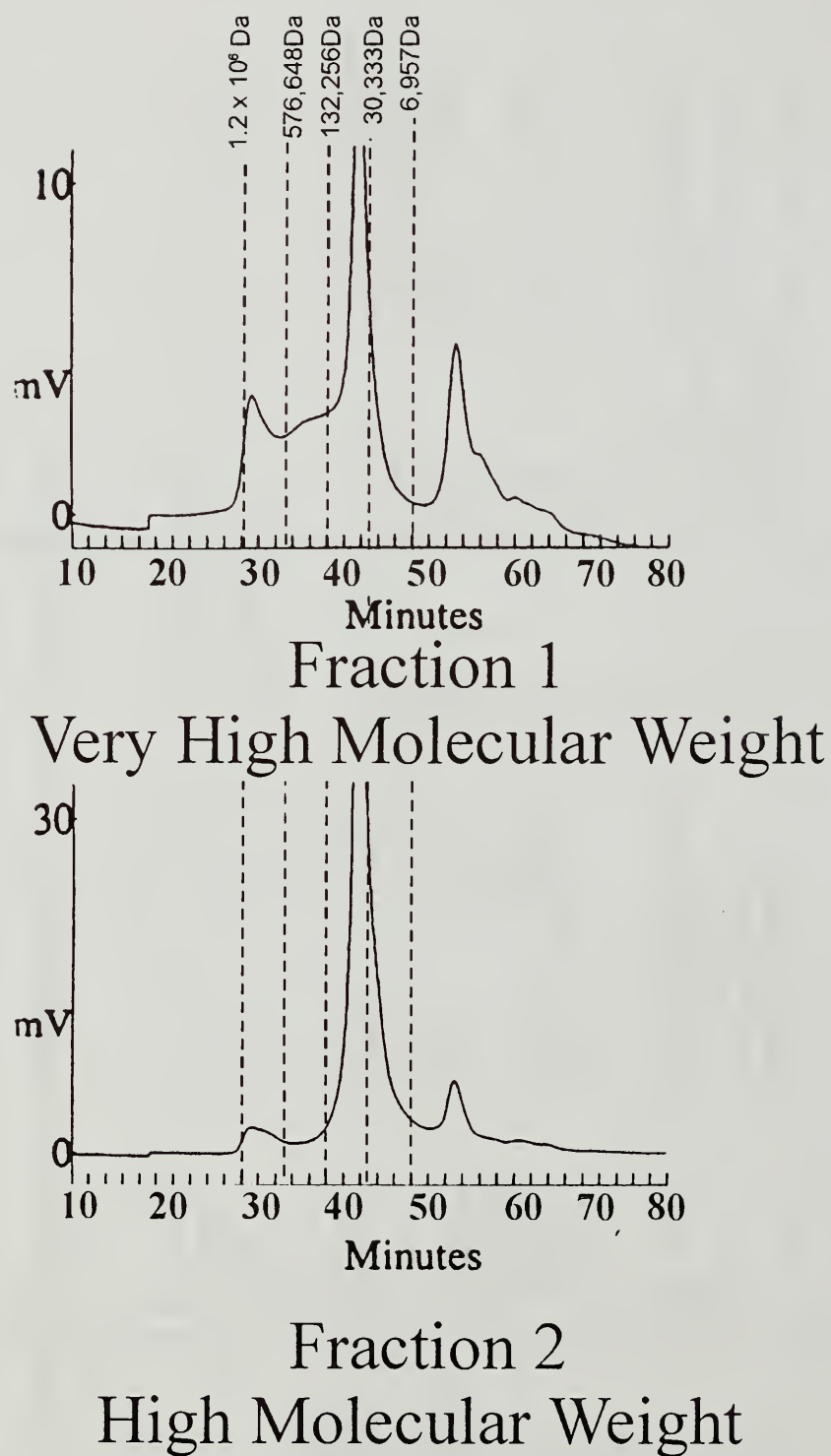
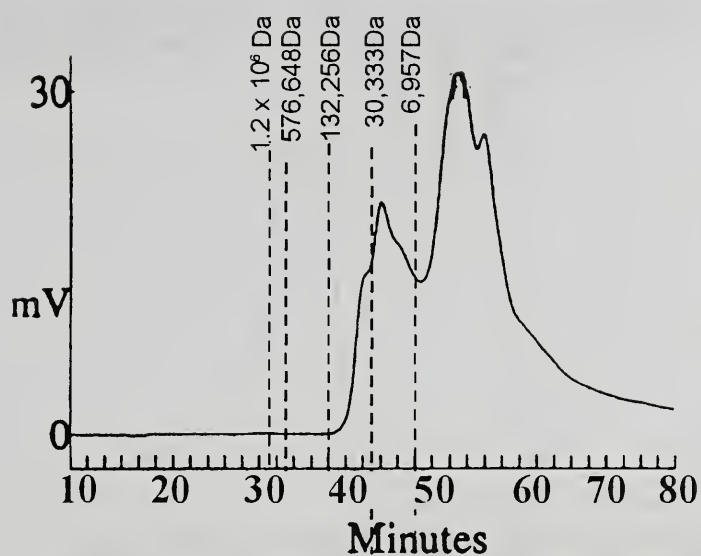
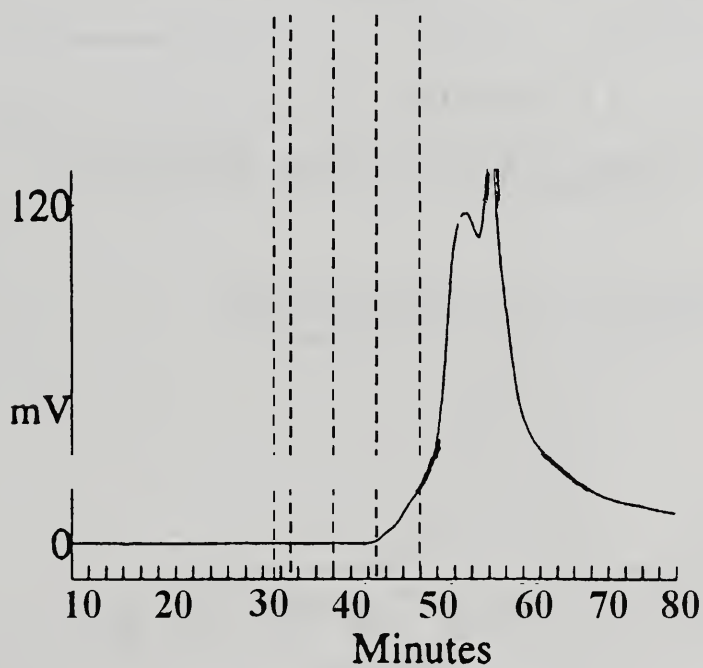


Figure 6. Retentate cane final C molasses on gel filtration - Fraction 1 and 2.

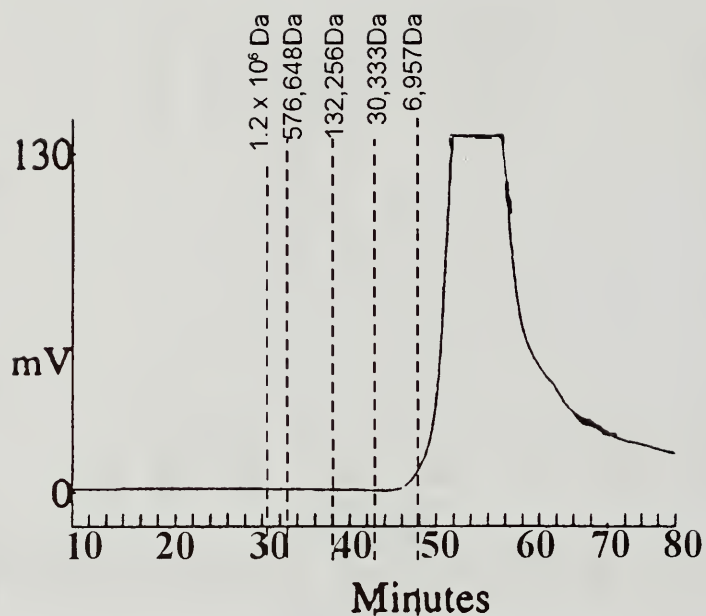


Fraction 3 Medium Molecular Weight

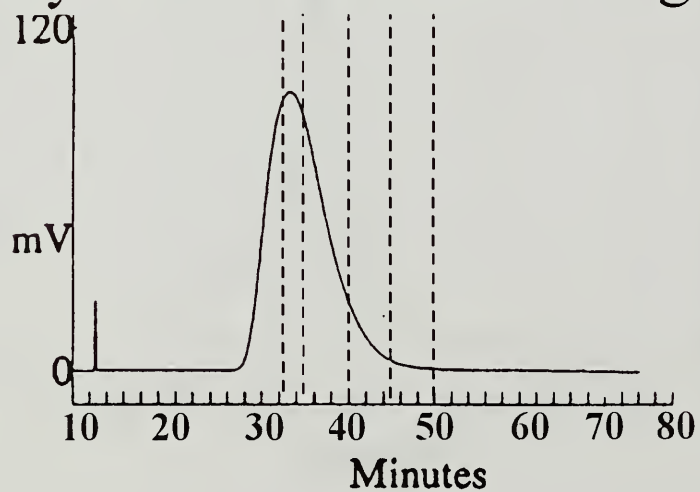


Fraction 4 Low-Medium Molecular Weight

Figure 7. Retentate cane final C molasses on gel filtration - Fraction 3 and 4.

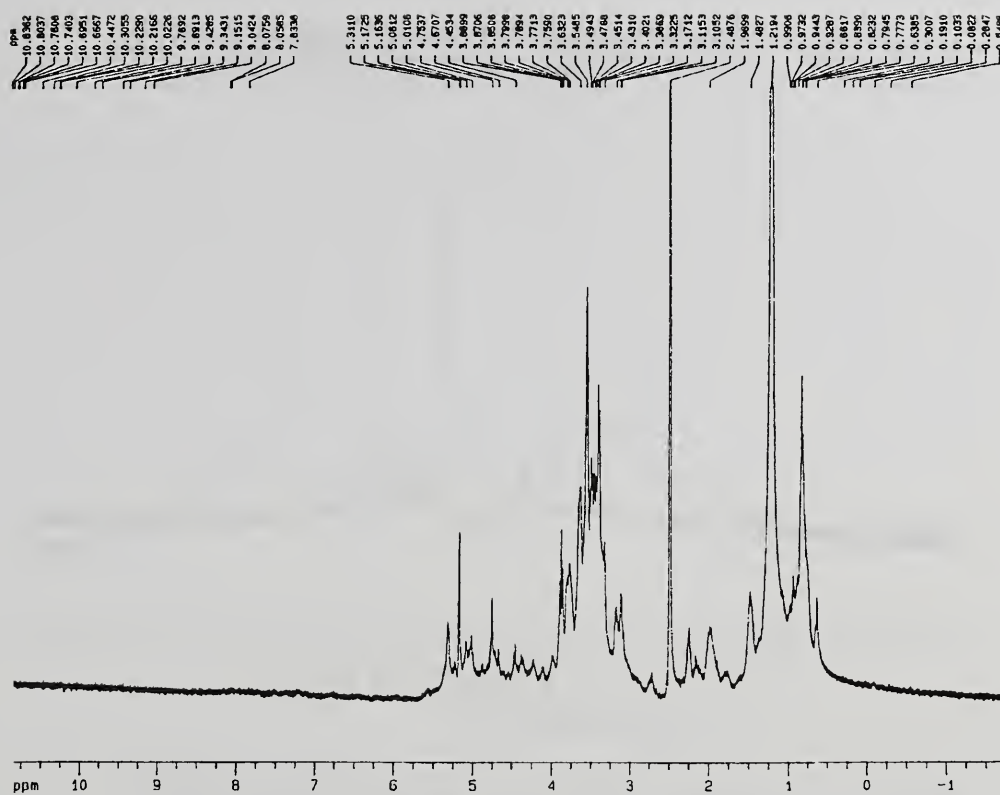


Fraction 5
Very Low Molecular Weight

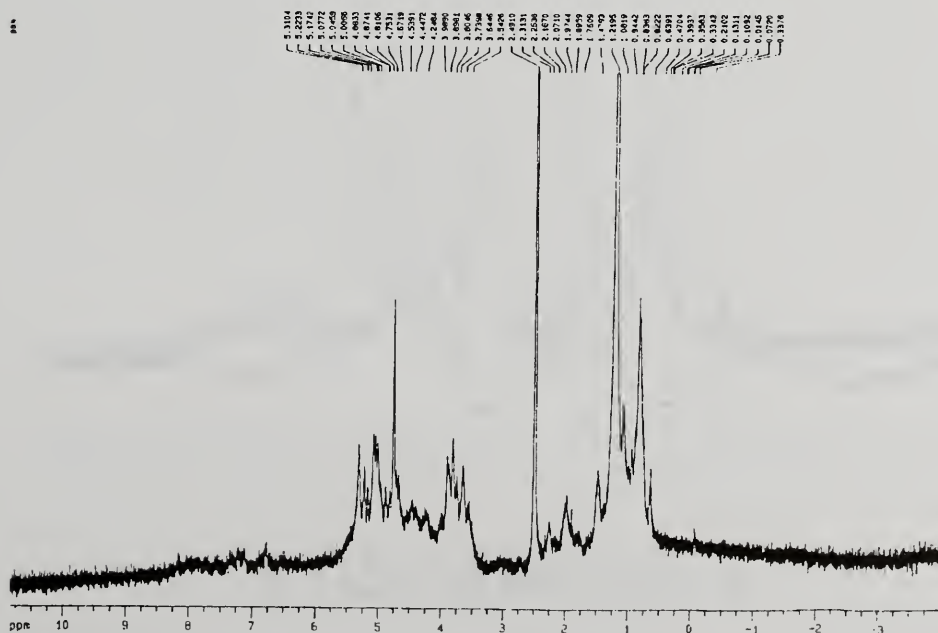


1.2MDa Standard

Figure 8. Retentate cane final C molasses on gel filtration - Fraction 5 and 6.



Fraction 1



Fraction 2

Figure 9. NMR - Fraction 1 and 2.

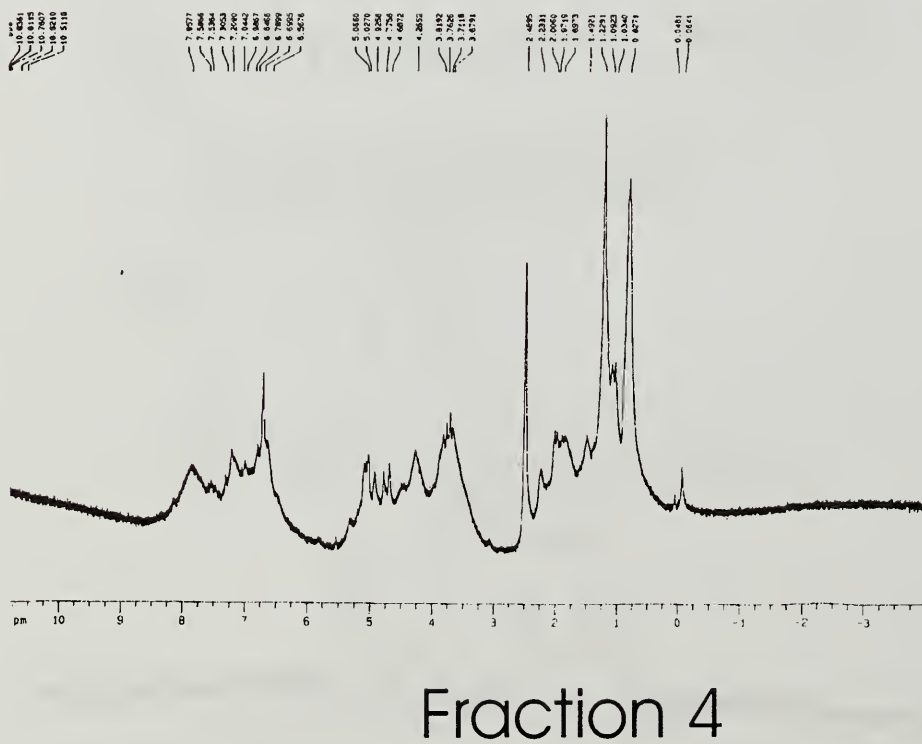
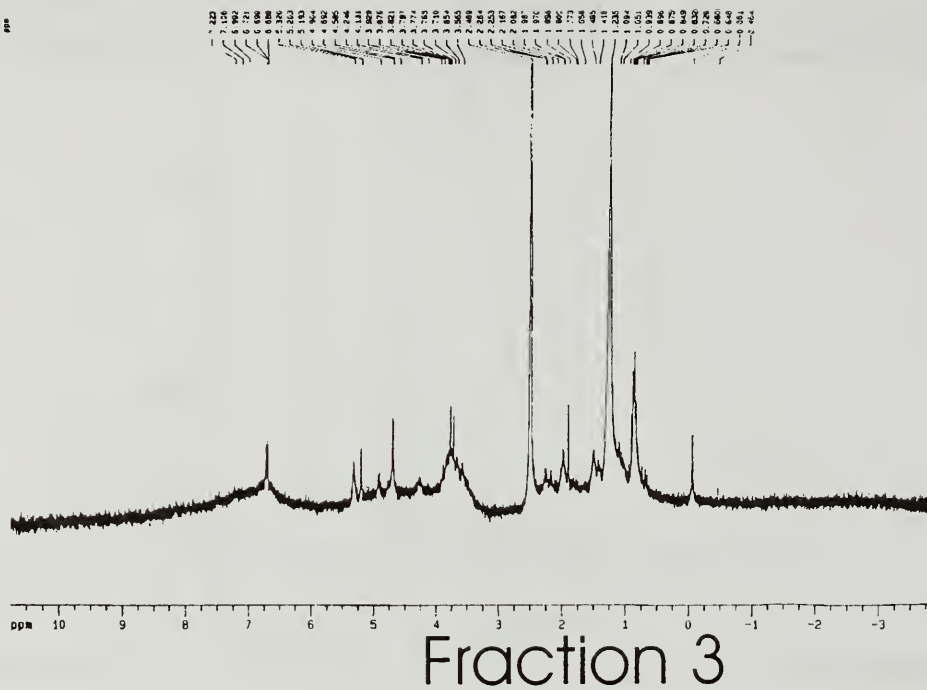


Figure 10. NMR - Fraction 3 and 4.

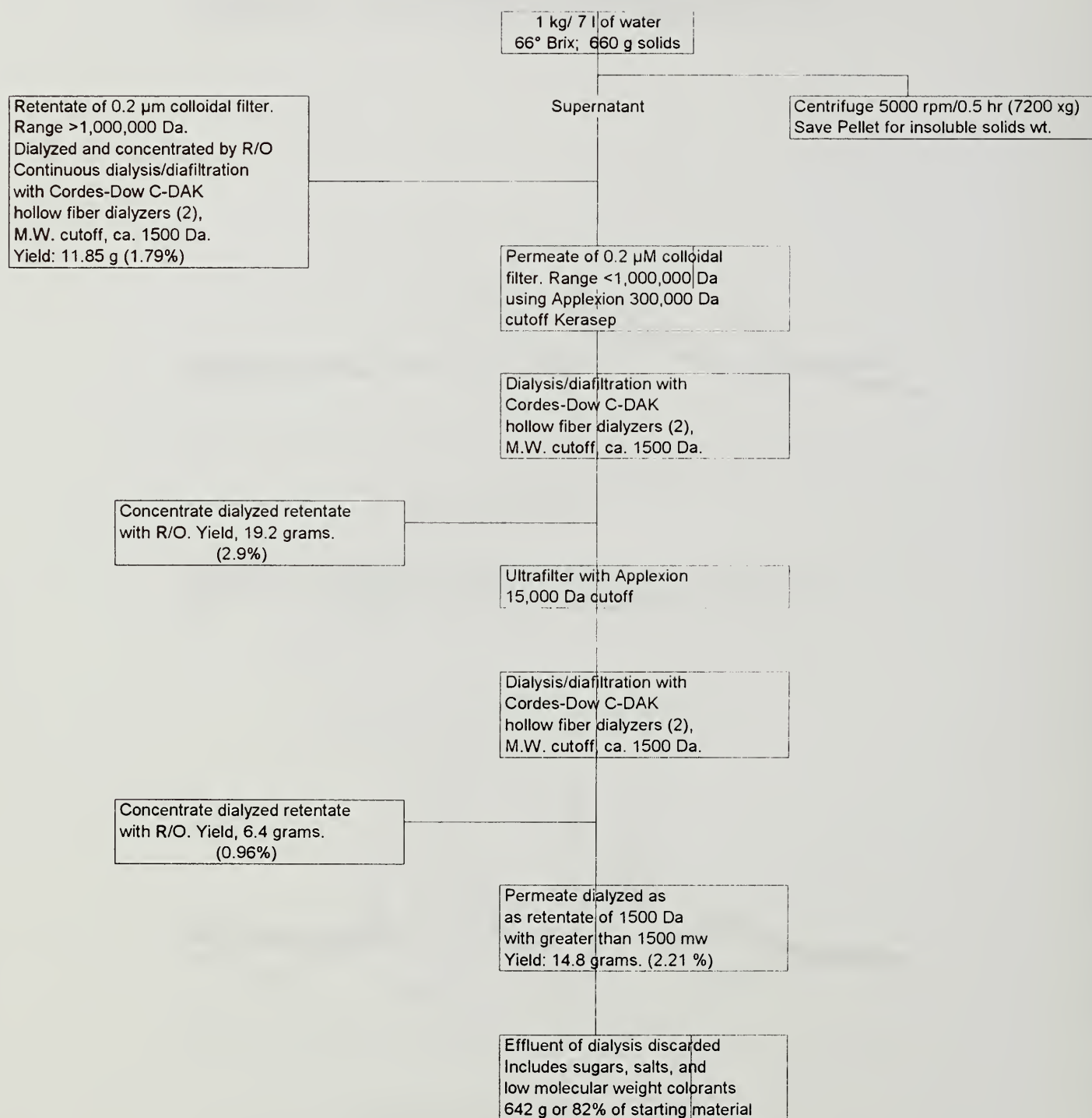


Figure 12. Ultrafiltration of sugarbeet molasses raffinate on Applexion Kerasep ceramic colloidal filters.

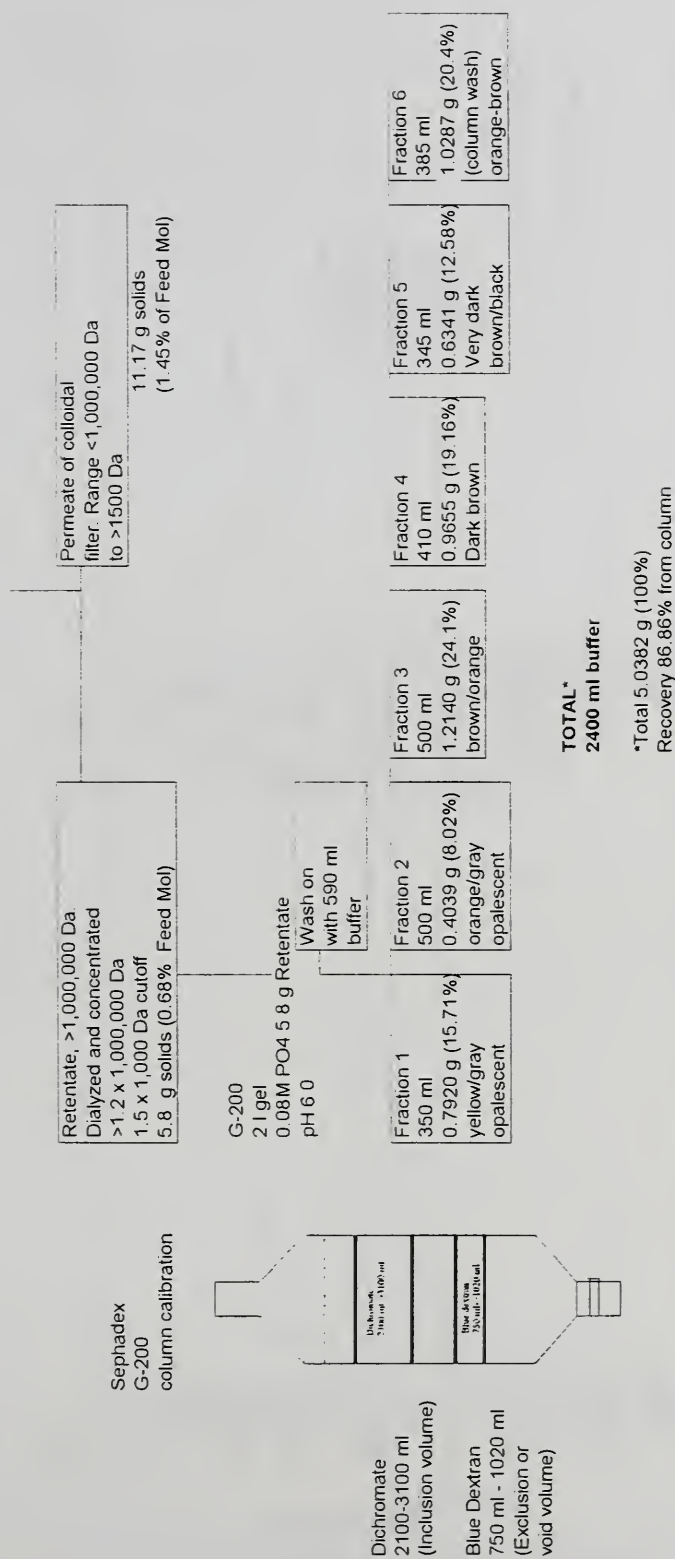


Figure 13. Preparative gel filtration of sugarbeet molasses colloidal retentate from ion exclusion raffinate.

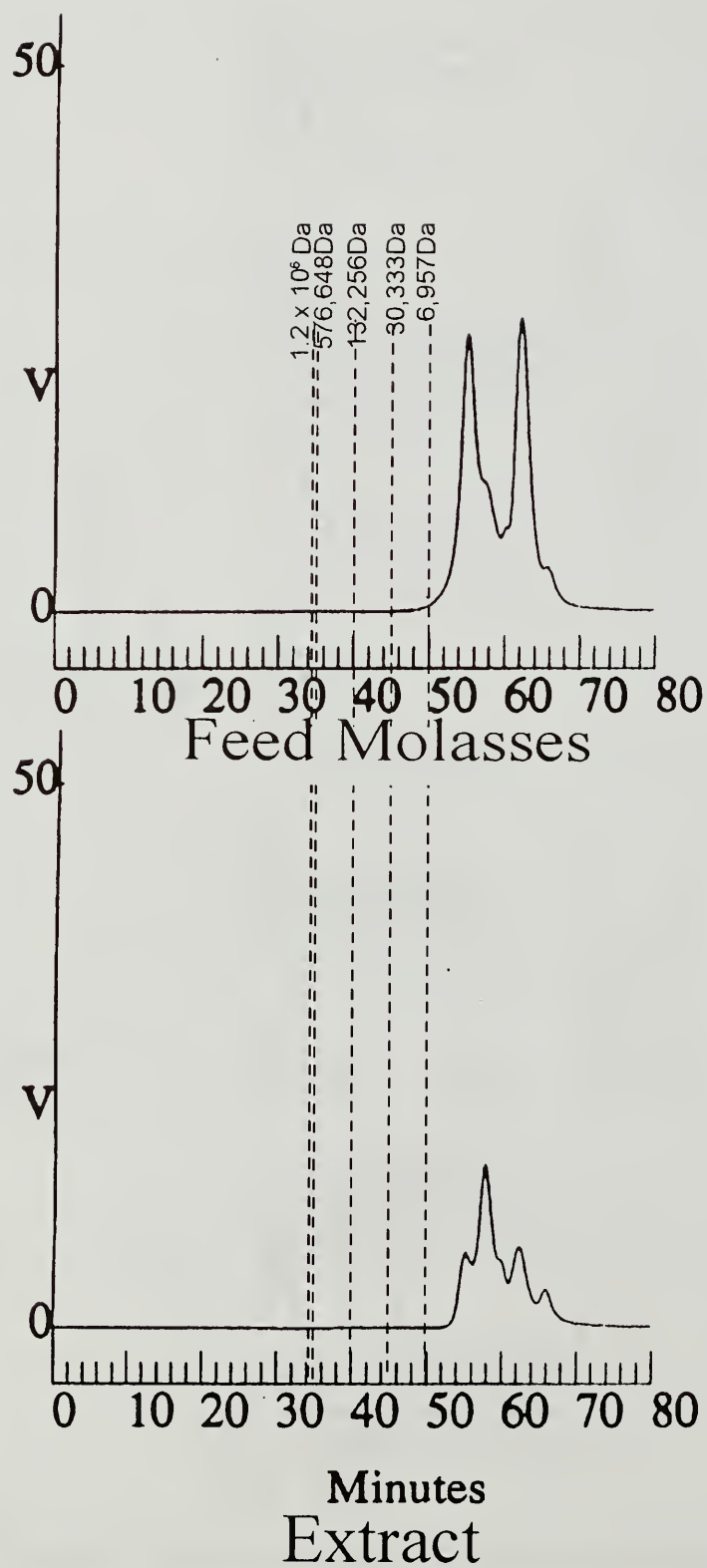


Figure 14. Ion exclusion of sugarbeet molasses - feed molasses and extract.

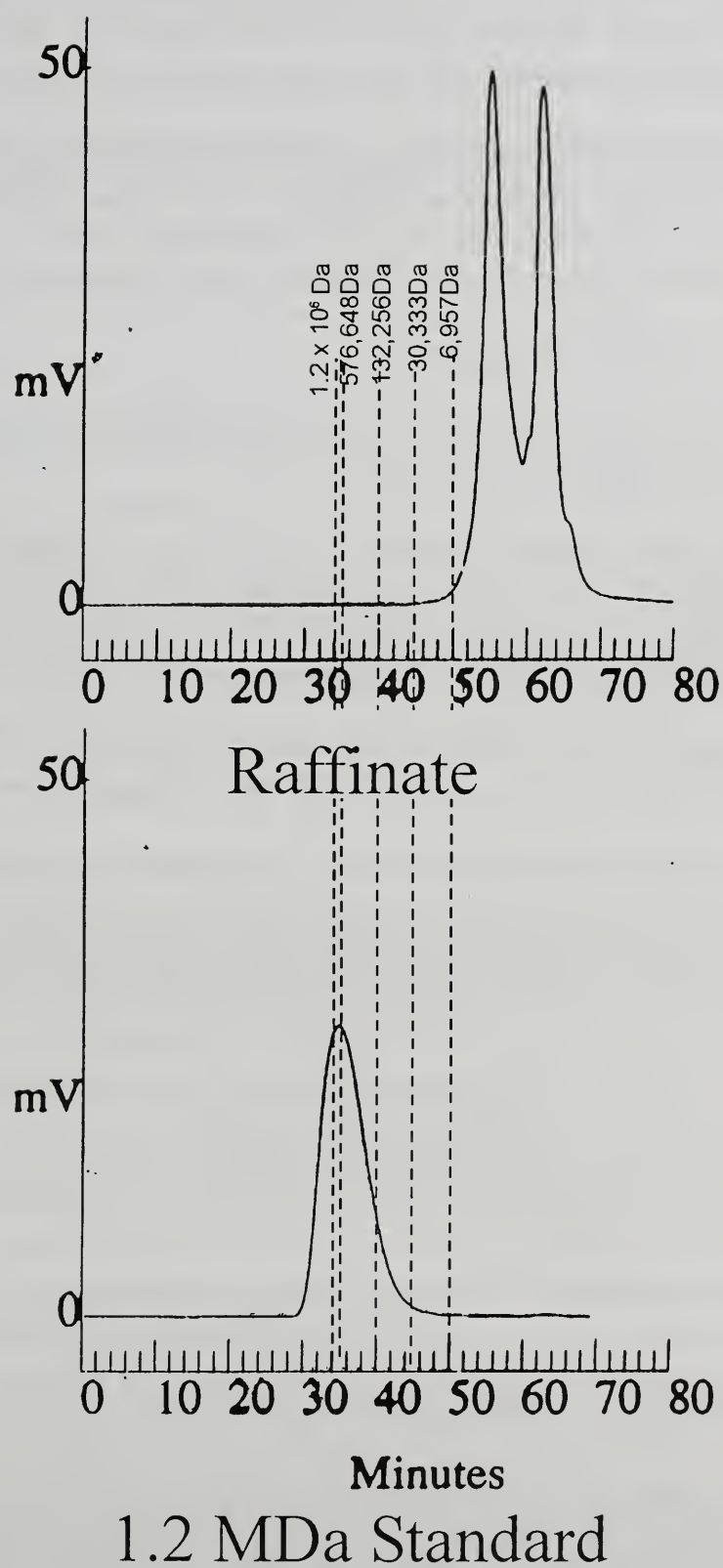


Figure 15. Ion exclusion of sugarbeet molasses - raffinate and 1.2 MDa standard.

DISCUSSION

Question: When you dialyzed your samples, did you monitor the permeate and analyze it to zero Brix?

Vercellotti: Yes. We monitored all of these separations by conductivity and by phenol-sulfuric acid assay. We use the latter to monitor carbohydrate going out. All of those dialyzates and permeates were also very closely monitored by UV absorption. We have concentrated down the final wash-out from the dialyzate. We can take a whole garbage can full of reverse osmosis output and concentrate it down to a liter and look at what is in it. Besides sucrose, there are a lot of salts, and all kinds of low molecular weight organics, particularly phenolic-type organics.

Question: Are you sure they are there because they just stayed behind the membrane, they were rejected by the membrane or they did not get washed out completely?

Vercellotti: That is a very good question. In the last part of the colloidal filtration, using a 0.2 μ membrane, we literally diafilter it. We get at a constant level - we keep adding water for several hours and we do the colloidal filtration exhaustively so that anything of lower molecular weight should come out, unless the pores are completely plastered over on the inner surface of the hollow fiber device.

We also do the C-DAK dialysis on these. I would think that the 1500 Da cut-off kidney dialyzer ought to get all that lower molecular weight material out, but it does not. The agglomerate is there.

Question: What was the purpose of the second test on your ceramic membranes? Was it basically to prove that membranes have very good molecular weight cut-off? In my opinion, dialyzing the product, basically anything that does not go through the membrane stays in the retentate, but it does not mean that all this material blocks the pores. A small portion of this material does affect permeation, but the rest of it does not.

Vercellotti: The first slide showing sugarcane final C molasses with a 15,000 Da molecular weight nominal cut-off ceramic membrane was to show that the high molecular weight material from sugarcane molasses is retained whether you are using an organic membrane or the ceramic membrane. That was just a comparison.

The last ultrafiltration sequence where a 0.2 μ ceramic membrane was used and a 300,000 Da molecular weight cut-off ceramic membrane and a 15,000 Da molecular weight cut-off ceramic membrane and a 1500 Da molecular weight cut-off dialyzer really did fractionate the raffinate from the ion exclusion process into four different categories. And these are rather different in their molecular properties.

Question: You showed that part of the substances that fouled the membrane are beet colorants. I have to disagree with this because if beet colorants are retained, you would see significant color reduction with this system. You do not see much color reduction across a 0.2 μ membrane. I think that there might be some other components that eventually block the membrane surface.

Vercellotti: I cannot disagree with you.

IDENTIFICATION OF ESTERIFIED PHENOLIC AND ORGANIC ACIDS IN THE HIGH MOLECULAR WEIGHT COLORANT/POLYSACCHARIDE FRACTION IN CANE SUGAR PROCESSING

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ABSTRACT

Plant cell walls, particularly those of grasses and grains, contain phenolic acid residues that are part of the lignin-polysaccharide network that strengthens the structure of the plant. Soluble cell wall material from the sugarcane plant comprises part of the high molecular weight complex of colorant and polysaccharide that is present throughout cane sugar processing, is implicated in color increase, and has an affinity for the sucrose crystal. Base hydrolysis is a well established method for releasing esterified ("base labile") compounds from polysaccharides. Base hydrolysis of the high molecular weight material from cane sugar processing materials released a high concentration of phenolic acids as well as a surprising number of organic acids. It has been speculated that some of these acids, which include di- and tri-carboxylic acids, may contribute to the cross linking of colorant molecules to the polysaccharide matrix. This study examines the base labile components in sugar processing as well as in cane varieties.

INTRODUCTION

A significant proportion of the colorant in cane sugar processing exists as high molecular weight (>10,000 Da) colorant-polysaccharide complexes (1,2,3,6,23). Clarke, *et al.*, (1) showed that a high molecular weight component with both visible color and polysaccharide characteristics was in the raw sugar crystal and proposed simple tests for its evaluation as a measure of raw sugar refining quality. The cross-linking of polysaccharides by polyphenolic bridges was recently proposed by S.P.R.I.

Some of the colorant-polysaccharide complex is persistent and difficult to remove in processing and, because of its carbohydrate nature, has a propensity to occlude within the crystal. The composition and structure of this type of colorant and the nature of the complex has been the subject of a long-term research study at S.P.R.I. (2,3,5,6). It has been proposed that colorant or colorant precursor is associated with polysaccharide molecules probably by covalent bonding.

Phenolics and polysaccharides are associated in plants in several ways. Phenolic monomers and dimers form a network to make up lignin, which is mostly insoluble. Phenolics, such as ferulic acid and cinnamic acids, also act as crosslinking agents in cell wall hemicelluloses (10,16,17,19). Ferulic acid and p-coumaric acid (p-hydroxycinnamic acid) are covalently bound to cells walls of the Gramineae, the family to which sugarcane belongs (12,14,22) and to many other plants also. Markwalder and Neukom (19) and Nevins and Kato (20) discussed how ferulic acid dimerizes to act as a crosslinking agent to stabilize arabinoxylan networks in corn (Figure 1). The crosslinking of phenolic compounds with polysaccharides or oligosaccharides to stabilize the cell wall structure is a universal mechanism in plants.

Lignin in grasses and cereals contains three related aldehydes, p-hydroxybenzaldehyde, vanillin (4-hydroxy-3-methoxybenzaldehyde) and syringaldehyde (4-hydroxy-3,5 dimethoxybenzaldehyde), which are released on mild alkaline oxidation (11). These three aldehydes were reported in cane bagasse lignin many years ago (4). p-Hydroxycinnamic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid and ferulic acid also were identified as esterified components released from cane bagasse by normal sodium hydroxide (21). Kato and co-workers (13), working with water-soluble lignin-carbohydrate complex from enzyme treated bagasse, showed the release of ferulic acid, p-hydroxycinnamic acid, and feruloylated tri- and tetrasaccharides therefrom.

The relevance of this to sugar production is that some of these phenol-polysaccharide-colorant complexes are soluble and are expressed into the juice, undergo many different reactions during processing, and remain in raw sugar, where they have to be removed during the refining process.

Today, phenolics are also interesting from a nutritional standpoint, because of their antioxidant ability and their potential in fighting disease. For example, the action of wheat bran as a potential anticarcinogen is related partly to the presence of phenolic acids esterified to the fiber, causing it to have a low fermentability in the large intestine (15). Almost every month, a new publication appears about the good antioxidant characteristics of foods such as tea, coffee and wine.

In this study, the base labile (hydrolyzable by base) compounds in fresh cane juice, raw sugars, mill processing samples, and white sugar were examined.

EXPERIMENTAL

Samples. The high molecular weight (>12,000 Da), nondialyzable material was isolated from the juice of nine cane varieties, from 6 raw sugars from different locations, from sugar mill processing samples, and from one white sugar by dialysis.

Fresh, clean cane juice was obtained in mid-December, 1997, from nine sugarcane varieties grown at the U.S.D.A. Sugarcane Research Unit at Houma, Louisiana. The juice was initially filtered through a cloth filter to get rid of soil and plant debris. After dialysis, the juice was filtered on No. 4 Whatman filter paper (coarse porosity) to remove the finer particles of extraneous material that had escaped the initial filtration.

Raw sugars were obtained from the store of raw sugars at S.P.R.I., and represented a variety of geographical locations. One freshly produced, first strike white sugar was also examined.

Mill process samples were obtained from a sponsoring company of S.P.R.I.

High molecular weight material (>12,000 Da) was isolated from the samples by dialysis for 100 hours through cellulose acetate tubing with the appropriate cut-off pore size. Water was removed by rotary evaporation under vacuum and the nondialyzable material (the tenate) was freeze-dried for future work-up.

Base hydrolysis. Base hydrolysis of materials was carried out according to the method of Kroon, *et al.* (15), which derives from the methods of Hartley and Morrison (10), using 1 N NaOH to hydrolyze the samples, followed by acidification and extraction with ethyl acetate to obtain the phenolic acids and other released compounds.

In detail, the hydrolysis procedure was as follows: 200 mg of sample was dissolved in 20 ml 1 N NaOH and placed in a capped 125-ml glass flask in a water bath at 37°C for 24 hours, with gentle agitation. (As little as 20 mg of sample could be analyzed without difficulty.) The sample was allowed to cool to room temperature, and the pH adjusted to 2.5 with 4N HCl. The acidified hydrolyzate was extracted with ethyl acetate (3 aliquots of 25 ml each). The ethyl acetate extract was dried over powdered anhydrous sodium sulfate for at least 1 hour and up to overnight, filtered and concentrated to dryness. Dried sample was redissolved in pyridine containing the internal standard, trehalose, and converted to the volatile trimethylsilyl derivatives with Tri-Sil (Pearce).

GC/MS. Separation of constituents was done on a Hewlett Packard Series II Model 5890A gas chromatograph coupled with a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and a 70eV electron impact ion source. The column used was 30m x 0.25cm with 0.25µm film thickness, with a DB-5 crosslinked and bonded 5% phenyl 95% methyl silicone phase. Temperature program was 85°C for 4 min; increase 4°C per min to 100°C, then increase 10°C per min to 250°C for 10 min.

Compounds were identified on the basis of their mass fragmentation patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with a S.P.R.I. mass spectral library of standards.

RESULTS AND DISCUSSION

During neutralization and acidification of the alkaline solution of the tenates after base hydrolysis, the color of some of the cane varieties remained very dark after acidification, while others showed a marked decrease in color from dark brown to pale yellow. Figure 2 shows the juice color and ppm tenate in cane varieties. Dark and pale designations refer to the pH sensitivity of the tenates. The lack of pH sensitivity in some varieties seemed to correlate with varieties that had the highest juice colors.

The compounds identified in the ethyl acetate extracts of the hydrolyzates and their concentration in the tenates are shown in Table 1 for cane varieties, in Table 2 for the mill process samples, and in Table 3 for the raw sugar tenates.

The hydrolyzates are remarkable for the number and variety of compounds identified. These included 14 carboxylic acids, 17 phenolics, 23 fatty acids, and 7 other miscellaneous compounds. The same compounds were found in the fresh cane juice tenate through to the raw sugar, although in raw sugar, the amounts were reduced relative to the cane juice amounts. This is illustrated for several phenolic acids in Figure 3. *p*-Hydroxybenzoic acid, vanillic acid and syringic acid decreased by about 10 times (averaged results); *p*-hydroxycinnamic acid decreased by about two-thirds; and ferulic acid decreased about half. This change

in proportion reflects the selective removal of a portion of the original cane juice material in processing stages.

The base hydrolyzed tenate of the white sugar contained 6 phenolic acids (benzoic acid, p-hydroxybenzoic acid, vanillic acid, syringic acid, p-hydroxycinnamic acid and ferulic acid), 7 fatty acids (pentanedioic acid, decanoic acid, 2-hydroxypentanedioic acid, nonanedioic acid, palmitic acid, linoleic acid, and stearic acid), and two plant-derived carboxylic acids (succinic and fumaric). No aconitic, malic or citric acid was noted. The identified compounds represented 0.43% of the total weight of the tenate. The tenate in this sugar was 1416 ppm on sugar (0.14%), so these acids constitute about 6 ppm on sugar.

In some work preliminary to this study, several tenates from cane varieties and mill processing samples were tested for the presence of unbound material. This was done in two ways: (1) Whole, unhydrolyzed tenate was stirred with pyridine and silylated for GC/MS analysis. Small, unbound molecules would become evident with this treatment. (2) The tenate was dissolved in water, acidified to pH 2.5 and extracted with ethyl acetate. The extract was worked up in the manner described above and the extracted free acids analyzed by GC/MS.

The results of these two extraction procedures consistently showed that aconitic acid, citric acid, quinic acid, malic acid and stearic acid were not covalently bound to the tenate, but rather were released by solvents. However, pyridine alone was not usually enough to recover all of the aconitic, citric and stearic acids. They had to be extracted from an acidified solution with solvent. Usually, about the same amount or slightly less of these acids was recovered from the base hydrolyses, indicating little degradation. The fact that the same amount was recovered prior to base hydrolysis indicates no covalent binding of these acids to the polysaccharide matrix of the tenate.

However, the fact that not all of the material could be recovered with pyridine alone, shows that some sort of interaction had occurred, such as electrostatic attraction or hydrogen bonding, which was inactivated by the acid solution.

The fatty acids, palmitic, linoleic and oleic appeared to be partially free (loosely bound) and partially more tightly bound (released on base hydrolysis). About twice as much of these appeared on hydrolysis compared to simple solvent extraction of whole tenate.

The adsorption of the above acids may occur during juice extraction or as a consequence of the isolation procedures, either dialysis or ethanol precipitation, which also showed the same phenomenon.

Figure 4 illustrates the presence of aconitic acid and fatty acids in cane juice and raw sugar tenates. Although these acids are not covalently bound to the polysaccharide matrix, they do have a consistent affinity for the colorant complex throughout processing. Aconitic acid is especially interesting in this regard.

In an earlier study by Godshall and Grimm (7), hydrolysis of cane juice tenates from several varieties showed significant quantities of aconitic acid and leucoanthocyanidin. Separation of the tenates by gel permeation chromatography (GPC) showed three major high molecular weight peaks. Isolation and acid

hydrolysis of individual peaks showed that one peak (called Peak 3, about 300,000 Da in molecular weight) contained the very dark brown colorant, was high in leucoanthocyanidin pigment and had as much as 35% aconitic acid. This peak contained 50-60% carbohydrate; hydrolysis showed it to be high in mannose and glucose. This would indicate that although the aconitic acid is not part of the polysaccharide chain, as originally thought, it has a particular affinity for one fraction of the colorant-polysaccharide complex, remaining attached during 100 hours of dialysis and on separation by gel permeation chromatography and on desalting of eluent buffer with ion exchange resin.

As for phenolic compounds, the following were not found free in the tenates, only being released by alkaline hydrolysis: vanillic acid, vanillin, m-hydroxycinnamic acid, p-hydroxybenzaldehyde, syringaldehyde, isoferulic acid and sinapic acid. The following were sometimes found to be free in small but mostly insignificant proportions: p-hydroxybenzoic acid (proportion of free to bound = 1:10 to 1:100), syringic acid (1:10 to 1:100), p-hydroxycinnamic acid (1:100), ferulic acid (1:200), and caffeic acid (1:5).

None of the remaining fatty acids were found free in extracts of unhydrolyzed tenates. The number and variety of fatty acids was surprising, with saturated and unsaturated acids, alpha-hydroxy acids and dioic acids. Fatty acids from C6 to C20 were present. Benzoic acid derivatives conjugated with dicarboxylic acids (tartaric and malic) have been identified in alfafa (24). It may be possible to speculate that the dioic fatty acids could act as crosslinking and stabilizing elements.

The three major phenolic aldehydes found in lignin were identified in these samples -- p-hydroxybenzaldehyde, vanillin, and syringaldehyde (11). The structures are shown in Figure 5. The concentrations of each of these compounds in the cane juice varieties and in the raw sugar tenates is shown in Figure 6. Although the concentrations differed considerably in the cane juice from different varieties, the proportions were similar, with p-hydroxybenzaldehyde being the least concentrated and syringaldehyde the most concentrated. In the raw sugars, on the other hand, the proportions were different for each sugar. p-Hydroxybenzaldehyde was absent in the Peru sugar and syringaldehyde was much higher. Vanillin was the most concentrated in the Argentina and Zimbabwe sugars. p-Hydroxyacetophenone was also noted in these samples.

Dimeric phenolic acids have also been reported in cell walls of grasses (9,14,19). We were not able to identify any dimers.

However, two compounds with higher retention times had mass spectra very similar to vanillin and syringaldehyde, but with additional higher molecular weight fragments. These may be dimeric compounds. They were not detected in all samples. The spectra of these two compounds are shown in Figures 7 and 8.

Most of the phenolic acids identified as esterified to the high molecular weight colorant in cane have been found as free acids in raw sugar (8,18). The amount of free phenolics identified in fresh, clean cane juice is shown in Table 4 for three cane varieties. Free phenolics in raw sugar could be the result of preferential carry-over of free phenolics in the juice, as well as some alkaline hydrolysis that may occur in processing when pH is adjusted upward under processing temperatures.

As much as 3% of the total weight of the high molecular weight material in the cane juice varieties was released either by desorption or base hydrolysis. It should be noted, however, that base hydrolysis did not separate the bulk of the colorant from the polysaccharide, as after extraction with ethyl acetate, the color was essentially unchanged. The amount of color extracted by ethyl acetate was insignificant, in spite of the large concentration of phenolics.

The data for the cane mill samples, in Table 2, illustrate the changes that the colorant-polysaccharide complex can undergo in processing. It shows that several of the phenolics preferentially go into the raw sugar, including vanillic acid, m-hydroxycinnamic acid, p-hydroxycinnamic acid and ferulic acid. It also indicates that mill molasses is a rich source of this complex, with 3-4% on solids.

It should be noted that lactic acid, glyceric acid and glycolic acid are typical products of alkaline degradation of carbohydrates, which is most likely their source in these samples.

The large number of compounds associated with the high molecular weight colorant-polysaccharide complex in cane sugar processing, as it exists in the cane plant, bespeaks a very complex biology. The purpose for so many different phenolic and fatty acids is unknown.

SUMMARY AND CONCLUSIONS

1. The phenol-carbohydrate colorant complex that exists in the sugarcane plant continues, albeit altered, through processing into raw sugar and through refining, into white sugar. This is reflected in the different make-up of esterified phenolic and fatty acids in the tenates from various samples.
2. The presence of aconitic, citric, quinic and malic acids in the high molecular weight cane material is mostly due to loose electrostatic attraction or hydrogen bonding, which may occur during juice extraction or as a consequence of the isolation procedures. These acids are released by solvents. However, based on GPC results in an earlier study (7), the aconitic acid preferentially absorbs onto one peak, which is the one containing the highest proportion of colorant and leucoanthocyanin.
3. Although significant concentrations of phenolics were released during base hydrolysis, the material from which it was extracted retained essentially all of its pigment.

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Table 1. Base hydrolysis of cane variety tenates ($\mu\text{g/g}$ Tenate) arranged according to chemical type.

Compound	RT, min	µg/ g tenate								
		CP72-370	CP65-357	85-845	LCP83-153	CP70-321	LCP82-89	CP79-318	86-454	85-384
ppm of tenate (on solids)		3424	2995	1638	2396	1626	1594	2023	1950	2121
juice color, I.C.		18,545	12,085	11,366	10,451	8,240	7,889	7,300	6,595	6,291
juice I.V.		4.29	4.47	6.42	5.35	5.16	4.99	5.83	4.56	6.41
color of acidified tenate		dark	dark	dark	dark	pale	pale	pale	pale	pale
Carboxylic acids (may originate in cane plant or from carbohydrate degradation)										
lactic acid	6.32	393	490	425	458	684	657	1248	320	2066
glycolic acid	6.72	644	954	687	633	839	610	918	410	834
2-OH-butanoic acid	8.25	61	24	61	14	32	74	n.d.	52	54
3-OH-butanoic acid	9.09	69	34	140	13	156	2037	104	252	105
malonic acid	10.14	522	869	527	1154	679	476	416	275	790
succinic acid	12.21	349	921	407	506	449	459	305	431	356
glyceric acid	12.63	266	308	233	254	314	272	475	152	360
fumaric acid	12.76	116	121	92	128	78	82	74	67	74
2-OH-propanedioic acid	13.58	204	313	128	41	431	228	324	236	429
2,4-di-OH-butanoic acid	13.89	41	52	38	39	18	48	42	34	47
aconitic acid	18.05	859	8858	1011	11,115	1841	1121	1193	1242	919
citric acid	18.92	n.d.	639	n.d.	313	n.d.	n.d.	n.d.	n.d.	n.d.

Table 1. (continued)

Compound	RT, min	µg/ g tenate								
		CP72-370	CP65-357	85-845	LCP83-153	CP70-321	LCP82-89	CP79-318	86-454	85-384
Phenolics (originate in the cane plant)										
benzoic acid	10.85	32	88	57	64	76	42	57	54	49
p-OH-benzaldehyde	13.15	112	102	83	86	75	47	57	27	33
p-OH-acetophenone	14.56	77	64	53	46	73	24	trace	39	20
vanillin	15.46	230	448	200	244	226	282	316	132	199
p-OH-benzoic acid	16.61	684	574	524	419	521	510	415	223	379
syringaldehyde	17.50	350	614	519	474	352	801	550	263	340
vanillic acid	18.19	923	1119	887	500	534	807	850	398	643
m-OH-cinnamic acid	18.49	n.d.	n.d.	n.d.	n.d.	61	trace	trace	531	trace
syringic acid	19.60	729	1200	810	1098	775	1357	992	513	953
isoferulic acid	19.81	103	102	128	105	204	235	133	244	115
p-OH-cinnamic acid	19.96	209	264	149	173	216	302	150	162	195
3,4-dimethoxycinnamic acid	20.77	39	38	52	20	171	trace	63	n.d.	26
ferulic acid	21.44	344	479	346	359	542	648	453	578	315
vanillin-like phenolic	21.67	76	54	119	23	96	140	64	206	56
caffeic acid	21.88	trace	40	48	trace	91	30	trace	trace	trace
sinapic acid	22.77	83	145	72	85	133	161	77	98	104
syringaldehyde-like phenolic	22.89	64	53	208	25	137	170	33	145	48

Table 1. (continued)

Compound	RT, min	µg/ g tenate								
		CP72-370	CP65-357	85-845	LCP83-153	CP70-321	LCP82-89	CP79-318	86-454	85-384
Fatty acids (originate in the cane plant)										
hexanoic acid	6.51	47	70	31	58	90	67	75	36	185
heptanoic acid	9.12	14	6	7	trace	16	trace	n.d.	25	10
octanoic acid	11.21	14	5	7	3	15	28	trace	17	37
nonanoic acid	12.89	trace	trace	trace	trace	trace	trace	trace	trace	trace
2-OH-heptanoic acid	13.11	112	101	83	86	75	140	57	133	163
pentanedioic acid	13.65	31	46	37	7	41	26	69	42	29
decanoic acid	14.36	trace	trace	n.d.	trace	trace	11	trace	33	6
2-OH-octanoic acid	14.69	34	4	21	6	16	57	n.d.	140	224
hexanedioic acid	15.06	trace	trace	29	trace	23	29	trace	n.d.	trace
2-OH-pentaneodioic acid	16.04	26	54	36	10	44	42	30	36	35
dodecanoic acid	16.80	20	78	trace	trace	3	21	85	50	trace
octanedioic acid	17.40	112	119	108	100	49	164	92	164	182
nonanedioic acid	18.59	764	684	580	698	668	883	504	266	859
tetradecanoic acid	18.98	100	69	86	63	134	117	86	69	44
decanedioic acid	19.47	28	20	trace	18	31	27	23	33	19
palmitoleic acid	20.73	36	52	n.d.	trace	50	80	103	154	44
palmitic acid	20.97	3420	3408	2213	1613	3222	3654	969	3112	3673
2-OH-sebacic acid	21.07	285	264	218	232	313	326	173	356	331
heptadecanoic acid	21.80	33	27	28	8	62	38	28	29	88

Table 1. (continued)

Compound	RT, min	µg/ g tenate								
		CP72-370	CP65-357	85-845	LCP83-153	CP70-321	LCP82-89	CP79-318	86-454	85-384
linoleic acid	22.46	1815	2657	945	726	1220	1370	190	1136	1197
oleic acid	22.51	1644	1529	945	363	827	1071	82	1317	1251
stearic acid	22.70	931	724	795	512	1173	993	619	908	844
eicosanoic acid	24.60	38	35	31	31	27	42	49	124	65
Miscellaneous components (various origins -- cane plant or carbohydrate degradation)										
phosphoric acid	11.63	23	15	20	trace	19	trace	13	18	28
glycerol	11.65	70	132	103	109	171	223	13	164	250
RSP (m/z 145)	12.07	67	74	201	73	179	382	81	137	98
HMF degn (m/z 271)	14.84	43	41	67	12	67	107	49	81	72
pyrrolidinecarboxylic acid	15.34	51	92	66	66	111	96	100	100	96
quinic acid	19.42	120	157	124	117	152	93	79	104	55
cetyl alcohol	20.08	28	13	28	27	55	44	13	22	32
Total ppm on tenate		17,619	29,646	14,966	23,435	18,856	21,921	13,061	16,028	19,615

Table 2. Base hydrolysis of mill samples, ppm on tenate ($\mu\text{g/g}$ tenate) arranged according to chemical type.

Compound	RT, min	$\mu\text{g/g}$ tenate							
		Crusher	Residual	Dilute	Clarified	Raw	A Mol	B Mol	Final Mol
ppm tenate (on solids)		13,800	45,500	50,300	8,300	1,700	38,300	39,000	31,200
Carboxylic acids (may originate in cane plant or from carbohydrate degradation)									
lactic acid	6.32	363	750	366	306	307	417	486	569
glycolic acid	6.72	100	490	510	615	566	351	849	789
2-OH-butanoic acid	8.25	23	65	26	38	25	10	26	32
3-OH-butanoic acid	9.09	171	245	166	74	193	60	137	100
glyceric acid	12.63	21	113	146	113	91	41	153	177
malonic acid	10.14	24	132	72	112	53	18	46	133
fumaric acid	12.76	30	72	95	97	99	66	144	327
succinic acid	12.21	102	298	315	290	241	409	333	324
2-OH-propanedioic acid	13.58	n.d.	99	39	83	n.d.	n.d.	5	63
2,4-di-OH-butanoic acid	13.89	n.d.	n.d.	8	n.d.	n.d.	n.d.	n.d.	n.d.
3,4-di-butanoic acid	14.16	n.d.	n.d.	20	24	26	9	22	6
malic acid	14.95	18	105	158	180	114	65	117	122
aconitic acid	18.05	18	46	1967	206	4469	580	962	206
citric acid	18.92	n.d.	36	83	40	54	51	33	33

Table 2. (continued)

Compound	RT, min	µg/g tenate							
		Crusher	Residual	Dilute	Clarified	Raw	A Mol	B Mol	Final Mol
Phenolics (originate in the cane plant)									
benzoic acid	10.85	21	21	26	16	19	16	19	21
p-OH-benzaldehyde	13.15	5	35	20	19	24	5	9	10
p-OH-acetophenone	14.56	2	trace	13	10	trace	5	16	25
vanillin	15.46	13	63	107	93	61	38	92	52
p-OH-benzoic acid	16.61	36	106	165	136	71	145	132	132
syringaldehyde	17.50	13	19	115	109	93	60	65	47
vanillic acid	18.19	42	129	206	188	272	166	164	166
m-OH-cinnamic acid	18.49	n.d.	68	n.d.	98	316	38	93	86
syringic acid	19.60	58	89	267	230	126	168	174	196
isoferulic acid	19.81	32	129	223	279	129	271	220	231
p-OH-cinnamic acid	19.96	159	270	284	227	980	246	238	236
3,4-dimethoxycinnamic acid	20.77	n.d.	trace	trace	trace	trace	n.d.	trace	n.d.
ferulic acid	21.44	128	291	531	592	635	622	676	713
vanillin-like phenolic	21.67	28	trace	72	98	35	117	46	n.d.
caffeic acid	21.88	10	trace	trace	35	trace	trace	n.d.	n.d.
sinapic acid	22.77	n.d.	trace	n.d.	trace	n.d.	68	n.d.	12

Table 2. (continued)

Compound	RT, min	$\mu\text{g/g}$ tenate							
		Crusher	Residual	Dilute	Clarified	Raw	A Mol	B Mol	Final Mol
syringaldehyde-like phenolic	22.89	n.d.	n.d.	39	65	n.d.	93	43	n.d.
Fatty acids (originate in the cane plant)									
hexanoic acid	6.51	149	134	34	40	28	23	30	16
heptanoic acid	9.15	n.d.	12	10	7	trace	4	7	8
octanoic acid	11.21	58	51	15	19	10	16	19	16
nonanoic acid	12.89	38	51	7	trace	trace	8	9	7
2-OH-heptanoic acid	13.11	51	69	20	38	24	6	18	11
pentanedioic acid	13.65	4	30	10	14	19	3	8	32
decanoic acid	14.36	22	61	26	22	26	35	32	32
2-OH-octanoic acid	14.69	13	33	10	12	trace	trace	3	13
hexanedioic acid	15.06	8	15	9	15	20	4	7	9
2-OH-pentanedioic acid	16.04	4	21	17	24	24	7	17	13
dodecanoic acid	16.80	28	94	41	21	54	24	27	38
octanedioic acid	17.40	56	126	41	74	81	16	30	29
nonanedioic acid	18.59	429	611	206	195	315	115	93	87
tetradecanoic acid	18.98	57	183	69	94	89	69	65	68
decanedioic acid	19.47	22	31	12	18	23	17	9	12
palmitoleic acid	20.73	n.d.	trace	39	95	81	40	31	54

Table 2. (continued)

Compound	RT, min	$\mu\text{g/g}$ tenate							
		Crusher	Residual	Dilute	Clarified	Raw	A Mol	B Mol	Final Mol
palmitic acid	20.97	1981	3967	2997	2755	2318	1926	3466	4090
2-OH-sebacic acid	21.07	trace	250	34	102	83	100	24	80
heptadecanoic acid	21.80	46	262	121	69	44	96	63	75
linoleic acid	22.46	100	129	1304	2004	1054	1025	3256	4425
oleic acid	22.51	153	355	578	668	520	258	1085	1106
stearic acid	22.70	416	930	683	574	624	281	537	590
eicosanoic acid	24.60	66	228	94	128	100	69	97	86
Miscellaneous components (various origins -- cane plant or carbohydrate degradation)									
phosphoric acid	11.63	n.d.	n.d.	10	27	14	2	14	36
glycerol	11.65	n.d.	n.d.	41	54	55	19	28	17
RSP (m/z 145)	12.07	148	63	45	97	80	43	56	9
HMF degdn (m/z 271)	14.84	17	50	8	23	25	23	7	14
pyrrolidinecarboxylic acid	15.34	13	15	22	41	46	10	16	13
quinic acid	19.42	12	38	50	57	53	10	37	43
cetyl alcohol	20.08	15	32	21	38	32	10	8	13

Table 3. Base hydrolysis of raw sugar tenates, ppm on tenate ($\mu\text{g/g}$ tenate) arranged according to chemical type.

Compound	RT, min	$\mu\text{g/g}$ tenate					
		Argentina	Fiji	Florida	Peru	Trinidad	Zimbabwe
ppm tenate (on solids)		3150	1560	2131	1691	2292	3800
total ppm compounds on tenate		3,366	4,913	3,401	9,955	3,657	6,382
Carboxylic acids (may originate in cane plant or from carbohydrate degradation)							
lactic acid	6.32	109	472	85	124	156	112
glycolic acid	6.72	171	584	166	127	210	219
2-OH-butanoic acid	8.25	2	4	2	trace	2	2
3-OH-butanoic acid	9.09	2	15	5	12	13	3
malonic acid	10.14	114	79	16	20	16	11
succinic acid	12.21	182	294	42	64	101	104
glyceric acid	12.63	91	268	45	55	56	66
fumaric acid	12.76	162	37	195	86	154	148
2-OH-propanedioic acid	13.58	11	84	7	4	49	12
2,4-di-OH-butanoic acid	13.89	2	n.d.	n.d.	4	n.d.	n.d.
3,4-di-OH-butanoic acid	14.16	5	8	8	9	n.d.	3
malic acid	14.95	26	73	9	28	23	26
aconitic acid	18.05	715	667	167	5688	314	1117
citric acid	18.92	32	9	n.d.	53	trace	31

Table 3. (continued)

Compound	RT, min	μg/g tenate					
		Argentina	Fiji	Florida	Peru	Trinidad	Zimbabwe
Phenolics (originate in the cane plant)							
benzoic acid	10.85	4	6	8	n.d.	6	4
p-OH-benzaldehyde	13.15	3	8	6	18	5	8
p-OH-acetophenone	14.56	4	8	8	9	12	19
vanillin	15.46	20	15	8	11	10	17
p-OH-benzoic acid	16.61	34	60	38	34	71	86
syringaldehyde	17.50	7	23	14	67	15	14
vanillic acid	18.19	70	68	54	30	90	62
m-OH-cinnamic acid	18.49	21	n.d.	25	20	30	19
syringic acid	19.60	70	158	126	116	92	165
isoferulic acid	19.81	36	23	32	74	12	50
p-OH-cinnamic acid	19.96	58	183	81	91	245	214
3,4-dimethoxycinnamic acid	20.77	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
ferulic acid	21.44	63	211	13	400	167	377
vanillin-like phenolic	21.67	30	20	22	28	n.d.	18
caffeic acid	21.88	n.d.	n.d.	trace	trace	7	trace
sinapic acid	22.77	n.d.	17	16	27	31	49

Table 3. (continued)

Compound	RT, min	$\mu\text{g/g}$ tenate					
		Argentina	Fiji	Florida	Peru	Trinidad	Zimbabwe
syringaldehyde-like phenolic	22.89	6	9	11	31	10	6
Fatty acids (originate in the cane plant)							
hexanoic acid	6.51	7	trace	14	6	9	24
heptanoic acid	9.15	5	1	trace	trace	n.d.	5
octanoic acid	11.21	4	4	7	9	6	11
decanoic acid	14.36	3	5	6	4	4	7
nonanoic acid	12.89	trace	n.d.	n.d.	8	5	9
2-OH-heptanoic	13.11	9	8	18	19	10	25
pentanedioic acid	13.65	15	12	4	n.d.	4	7
2-OH-octanoic acid	14.69	trace	n.d.	trace	trace	trace	trace
hexanedioic acid	15.06	6	trace	8	17	5	6
2-OH-pentanedioic acid	16.04	22	31	3	8	11	12
dodecanoic acid	16.80	trace	trace	38	38	trace	82
octanedioic acid	17.40	19	32	33	29	42	74
nonanedioic acid	18.59	123	162	202	201	178	431
tetradecanoic acid	18.98	12	18	trace	37	trace	28
decanedioic acid	19.47	24	13	14	10	15	18

Table 3. (continued)

Compound	RT, min	$\mu\text{g/g}$ tenate					
		Argentina	Fiji	Florida	Peru	Trinidad	Zimbabwe
palmitoleic acid	20.73	9	trace	42	28	16	8
palmitic acid	20.97	408	685	998	1077	674	1475
2-OH-sebacic acid	21.07	36	60	n.d.	40	33	74
heptadecanoic acid	21.80	12	10	26	31	14	17
linoleic acid	22.46	262	126	153	452	203	137
oleic acid	22.51	156	190	356	445	203	547
stearic acid	22.70	72	86	174	125	141	294
eicosanoic acid	24.60	46	19	44	55	13	74
Miscellaneous components (various origins -- cane plant or carbohydrate degradation)							
phosphoric acid	11.63	3	n.d.	trace	n.d.	133	trace
glycerol	11.65	14	8	15	19	n.d.	15
RSP (m/z 145)	12.07	10	23	17	21	4	11
HMF degn (m/z 271)	14.84	11	3	3	5	17	5
pyrrolidinecarboxylic acid	15.34	trace	2	4	9	1	trace
quinic acid	19.42	19	12	7	18	13	10
cetyl alcohol	20.08	9	trace	6	14	6	14

Table 4. Compounds found in the free state in fresh cane juice (ppm on Brix).

Compound	CP 72-370	CP 65-357	CP 70-321
Carboxylic Acids			
Lactic Acid	22	24	19
Malonic Acid	71	110	54
Succinic Acid	29	48	26
Fumaric Acid	15	18	13
Malic Acid	707	1300	603
Aconitic Acid	34,300 (3.43%)	36,700 (3.67%)	25,200 (2.52%)
Citric Acid	1253	2030	1028
Phenolic Acids			
p-OH-Benzoic Acid	8	7	4
Quinic Acid	35	36	37
Syringic Acid	0	0	0
p-OH-Cinnamic Acid	17	20	22
Ferulic Acid	0	0	0
Cafferic Acid	0	0	0
Fatty Acids			
Palmitic Acid	124	164	131
Linoleic	18	16	19
Oleic	18	32	19
Stearic Acid	219	263	215

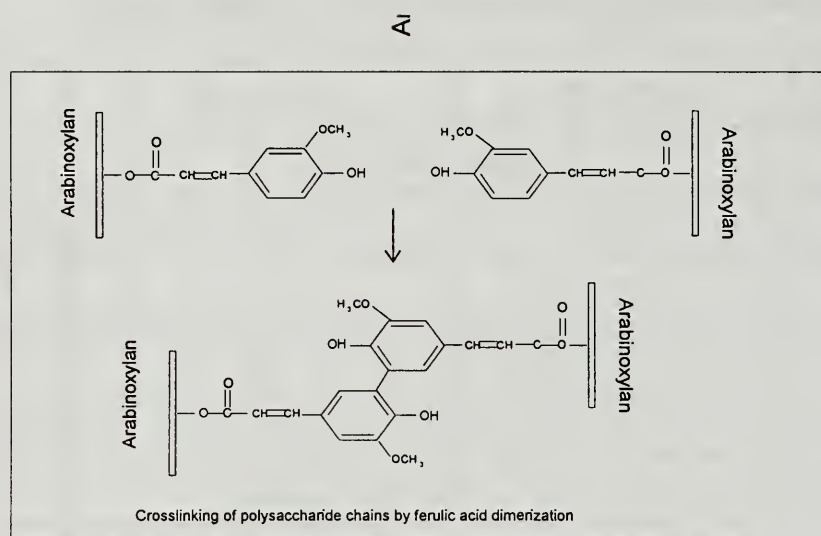


Figure 1. Ferulic acid dimerization and crosslinking of arabinoxylan chains to stabilize cell walls. (After Nevins and Kato, 1985, and Markwalder and Neukom, 1976.)

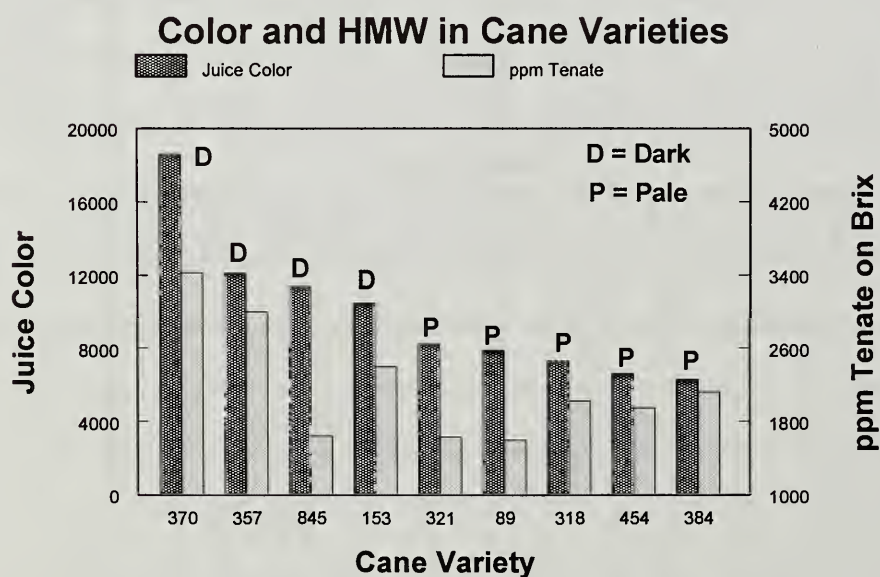


Figure 2. Juice color and ppm tenate in cane varieties. Dark and pale designations refer to the pH sensitivity of the tenates (ie, dark was not very sensitive to pH, and remained highly colored at acid pH.)

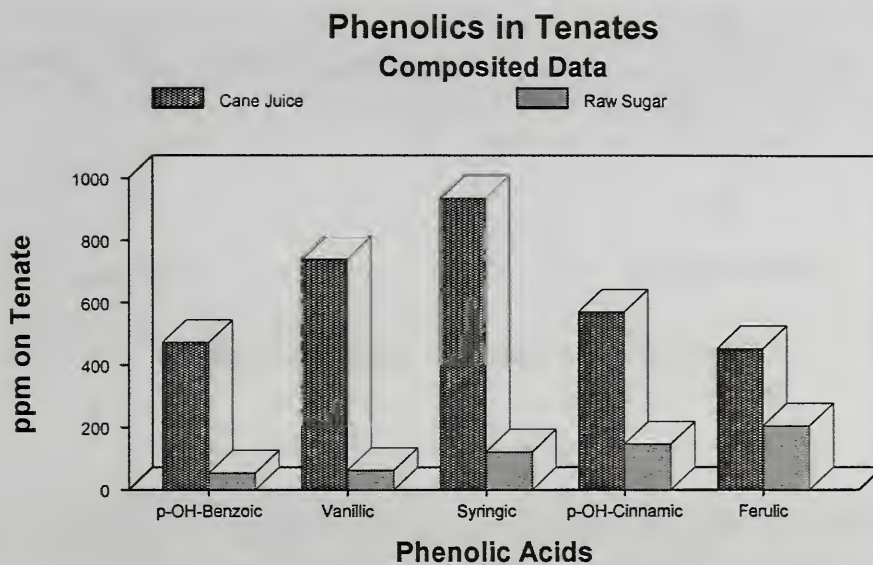


Figure 3. Comparison of phenolics in cane juice tenates and in raw sugar tenates. The phenolic acids are esterified to the polysaccharide matrix.

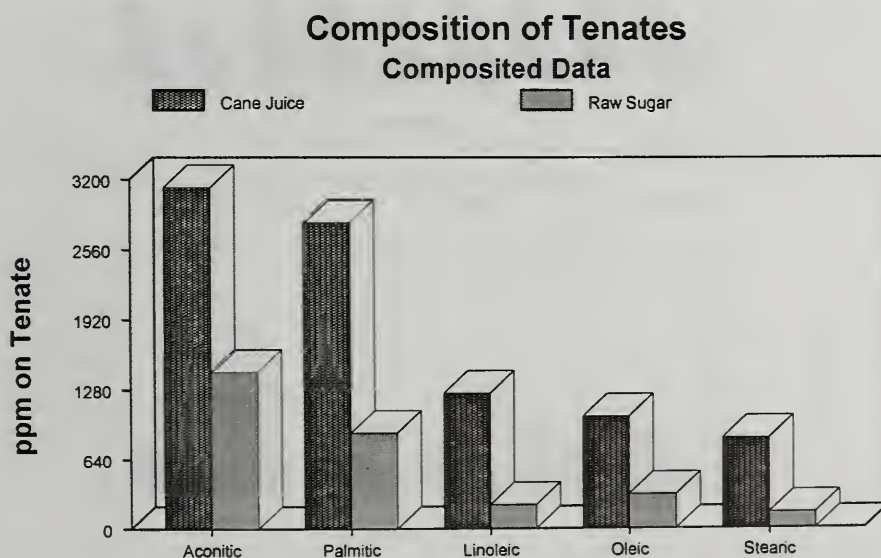


Figure 4. Comparison of aconitic acid and fatty acids in cane juice and raw sugar tenates. These acids are thought to be adsorbed to the polysaccharide-colorant complex and can be desorbed by solvents. They do, however, have a consistent affinity for the colorant complex throughout processing.

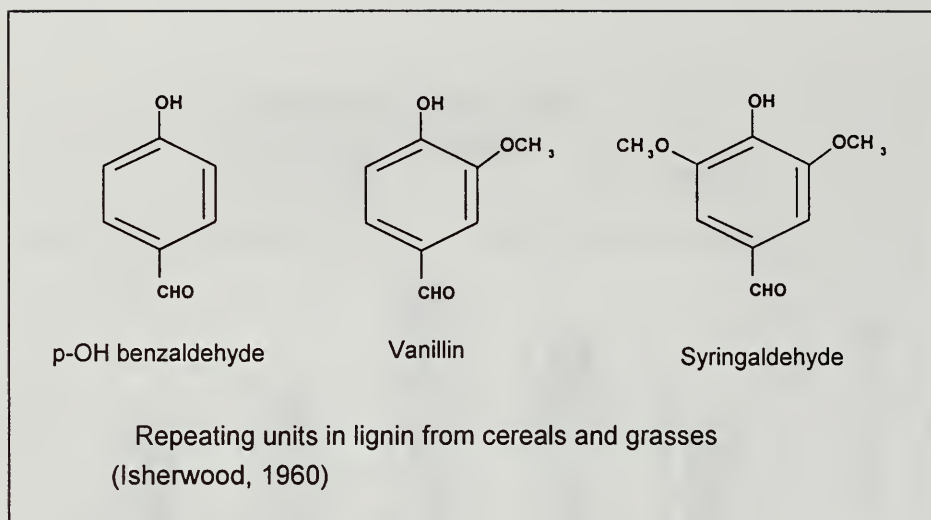


Figure 5. Phenolic aldehyde repeating units in lignin from cereals and grasses. All three of these were found in the base hydrolyzates of cane juice tenates as well as in raw sugar.

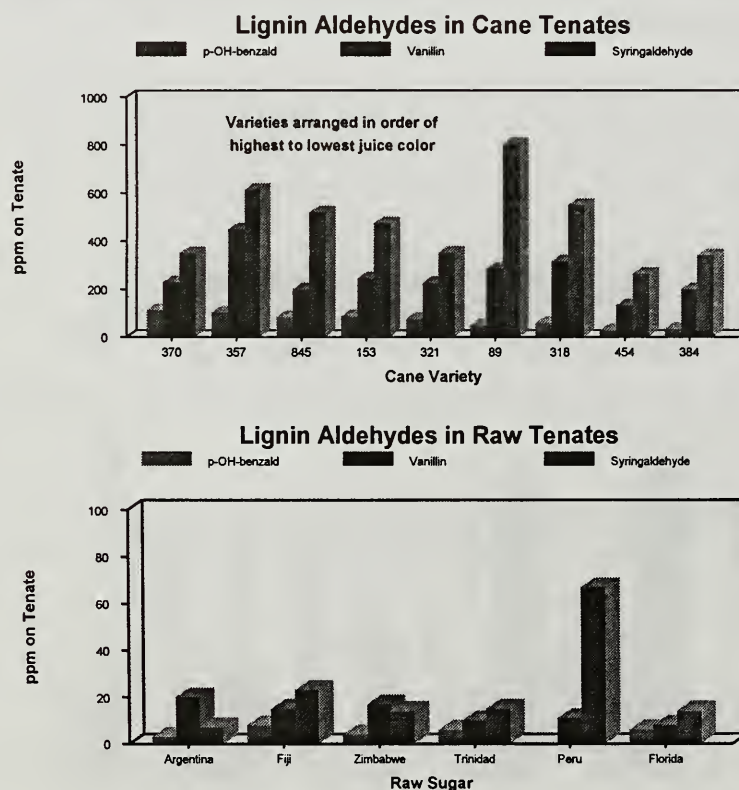


Figure 6. Comparison of lignin phenolic aldehydes in sugarcane varieties and in raw sugars.

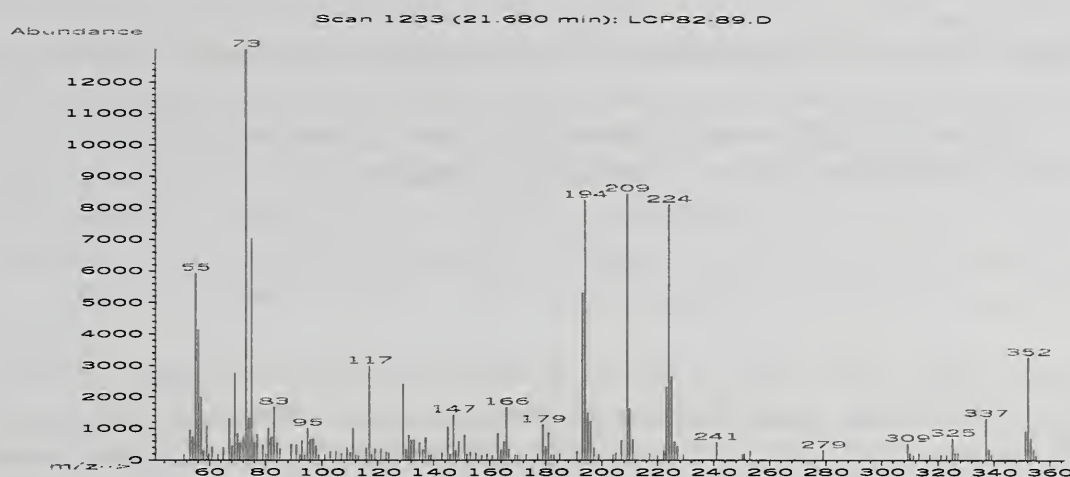


Figure 7. Spectrum of unidentified compound with vanillin-like characteristics but higher retention time and molecular weight. May be a dimer or bound to another phenolic or acid.

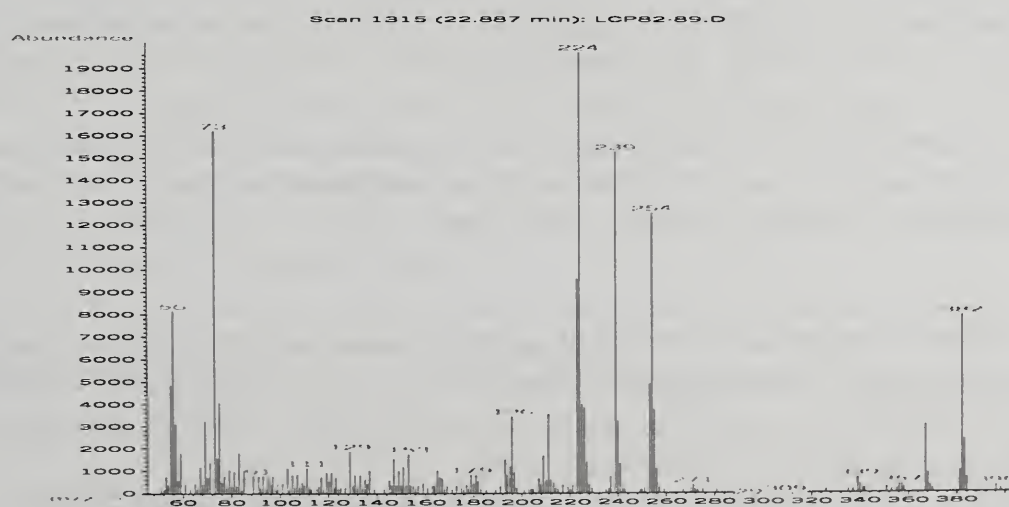


Figure 8. Spectrum of unidentified compound with syringaldehyde-like characteristics but higher retention time and molecular weight. May be a dimer or bound to another phenolic or acid.

USE OF SCANNING ELECTRON MICROSCOPE-ELEMENTAL ANALYSIS TO IDENTIFY SEDIMENTS IN SUGAR PROCESSING

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ABSTRACT

During processing, sugar often retains traces of insoluble materials that cause increased color or the appearance of turbidity or sediment as well as problems, such as filter blockage, during processing. These insolubles may be present in small quantities, and are difficult to identify. The scanning electron microscope (SEM) with an auxiliary energy-dispersive analysis system has provided a tool for studying these materials. The microscope provides magnifications great enough that the individual particles can be visualized. The energy-dispersive analysis system utilizes a spectroscopical x-ray analysis system to identify elements present in extraneous material. Analysis of elements, and the appearance of the particles provides a basis for identification of the material. Maps of elemental locations show the presence of specific elements in particles. The basis for use of this system for sugar insoluble analyses is presented, and examples of identification of various particles, with elemental spectra and dot maps are given.

INTRODUCTION

Insoluble solid material, known as sediment or turbidity, in sugars is the most common customer complaint from buyers of sugar (Dowling, 1988). The question of insoluble solids is therefore of considerable concern to all manufacturers of sugar—both final product white sugars and raw sugars. In raw sugar, insolubles adversely impact refinability by blocking filters, blinding membranes, depositing sediment at the head of filtration cisterns, increasing viscosity, and possible loss of sucrose to molasses. Sediment in white sugars adversely impacts product quality, causing, for example, a dull, unattractive appearance or haze in beverages.

The composition of haze, sediment and turbidity has been extensively studied from the point of view of causation of floc in beverages (Stansbury and Hoffpauir, 1959; Luizzo and Hsu, 1975; Roberts *et al.*, 1976; Clarke *et al.*, 1977), turbidity in clarified cane juice (Fort and Smith, 1954), in raw sugar (Devereux and Clarke, 1984) and refining (Hidi and McCowage, 1984).

Sources that typically contribute to the sediment and/or turbidity in sugar include equipment, such as rust, paint chips, and small pieces of metal; residues of processing aids, such as filter aid, carbon and resin beads; material from the plant or the field, such as starch granules, soil, clay and sand, waxes (from the cuticle of the cane plant, and insoluble matter in water), plant parts (pieces of bagacillo); spores or fungal filaments if microbial infection has occurred; and fibers and extraneous material that may come from outside sources not associated with the process.

The microscope is a powerful tool for the study of insoluble sediments in sugar, but is not in routine use (Cleriot, 1994).

Microscopes have been used for many years to structurally characterize various materials. The first microscopes were simple glass lenses that often provided remarkable information on microstructures. Monocular and binocular light microscopes became important scientific tools. In the mid-twentieth century a new type of microscope was developed that used electrons instead of light waves, and electromagnetic lenses rather than glass lenses. These electron microscopes, more specifically transmission electron microscopes (TEM), provided high resolution and high magnification. They require very thin specimens that are stable in the high vacuum of the microscope. Sample preparation is tedious and time consuming. The image in a TEM is formed when the electron beam passes through the sample and strikes a phosphorescent screen.

In the mid 1960's a new variation of the electron microscope became commercially available. This microscope, the scanning electron microscope (SEM), is similar to the TEM except that the electron beam does not pass through the sample, but scans the surface. This allows thick samples to be examined. The images provided are dimensionally relative to the sample, and are recognizable in general as magnified images of the sample. It is images from this microscope that most people recognize as electron micrographs.

Scanning electron images are formed from interactions of the electron beam of the microscope with the electrons in atoms of the sample. Figure 1 shows the many types of information generated from these interactions.

These electron beam interactions provide many possibilities for information retrieval, depending on the detector used. Three that are used regularly are: 1) transmitted electrons, 2) secondary electrons, and 3) x-rays. Transmitted electrons are those that pass through thin samples and are imaged in the TEM on a phosphorescent screen below the sample. They provide high resolution images and require ultrathin samples for examination.

Secondary electron images in the SEM are those formed using electrons that come from the sample itself, and are ejected from the sample surface. The beam scans the sample, and an image is formed on a cathode ray tube scanning simultaneously with the electron beam. The image formed is of the sample surface, therefore samples can be bulky rather than thin since the beam does not pass through the sample. Images are spatially related to the sample and correlate in the viewer's mind with the idea of what the sample should look like. For example, Figure 2 shows refined sugar crystals. Crystal structures are obvious, although most are imperfect. Figure 3 compares the appearance of turbinado sugar crystals. These crystals are larger and more perfect in crystal structure.

Such images provide information on the nature of the sample structure, and often can identify the material. However, if it were possible to know more about the composition of the sample, identification would be more likely. Referring to the electron beam information shown in Figure 1, one of the possible detection systems is x-rays. These x-rays are generated in the sample due to rearrangement of electrons in the atomic orbitals. They are emitted when the electrons in the orbitals shift to fill in lower energy orbitals from which an electron has been removed due to bombardment. X-ray generation is illustrated in the Figure 4. As electrons are removed from inner orbitals, those from outer orbitals move to more stable energy levels, and

x-rays of exact energy are emitted. The energies are specific to the element and to the electron level from which they are emitted. If the x-rays are collected and separated according to their energies, a spectrum is generated in which peaks correlate with energies from specific elements. Therefore, elements are identified by their spectral energies. A typical elemental spectrum is shown in Figure 5.

Additionally, by generating a dot map of areas from which the x-rays are produced, the location of specific elements in the sample can be shown. Comparing the secondary image to the x-ray dot map helps to identify the particles. The system has several limitations. These include failure to analyze light elements due to the inability of extremely low energy electrons to pass through the detector window. Systems with special windows are required for light element detection. Elements in sediments that are identical to those in the sample cannot be identified. For example, carbon cannot be detected in a carbon matrix. Additionally, x-ray energy peaks from higher energy orbitals of heavy elements may be identical to those of lower electron orbitals of light elements. When there are such energy overlaps, care must be taken in identification of the sample. In some cases small peaks are overlapped by stronger peaks and become lost. So, some spectra require intense interpretation if a complete analysis is required. Nevertheless, simple identifications can be made, and materials identified using the structural and elemental information available.

MATERIALS AND METHODS

Several procedures were used to obtain sediment samples from raw cane, refined cane and white beet sugar. These included:

Centrifugation. 100 g sugar at 50 Brix concentration was centrifuged 2500 rpm for 15 minutes. The resulting pellet was resuspended in water and recentrifuged 5 times to remove residual soluble material. The pellet was dried with 80% ethanol.

Filtration. 100 g sugar at 50 Brix concentration was filtered through a 0.45 μ Millipore membrane. The membrane was rinsed twice with 80% ethanol.

Dialysis. 400 ml of 50 Brix sugar solution was dialyzed through 12,000 MW cut-off cellulose membrane for 100 hours with toluene-saturated deionized water. Nondialyzable material was concentrated and freeze-dried.

SEM-EDX Sample Preparation. The filtered samples were directly mounted to a stub used for Scanning Electron Microscopy, secured with double sided tape. Samples prepared by centrifugation and those prepared by dialysis were resuspended with water, centrifuged or allowed to settle in a test tube. The resulting pellet was mounted onto a stub. The stub was subsequently air dried and coated with carbon. The sample was scanned under the Cambridge S-250 SEM and observed on the Tracor Northern 400 EDX system. The EDX provides elemental spectral analysis and elemental localization via analog Dot-mapping acquisition. The SEM magnification range was set between 30x-2000x. The elemental information was gathered by analyzing three separate, random areas of the SEM stub on the Tracor Northern 400 EDX system. All photographic images were recorded on Polaroid Type 55 P/N film.

The next several figures illustrate how microscopic and elemental information can be combined to characterize insoluble particles in various sugars. They are intended to show how the analysis system can be used to identify insoluble particles and therefore aid in solving a particular problem.

RESULTS AND DISCUSSION

For the purposes of this study, the most effective isolation technique was centrifugation. Dialyzed samples contained large quantities of high molecular weight soluble material (polysaccharides) which tended to coat and obscure the insoluble material. The background of the membrane filters tended to interfere with analysis of the trace amounts.

Figure 6 represents a very simple application, showing what appears to be diatoms. The x-ray spectrum shows one major peak, silicon. The dot map indicates a strong concentration of silicon in the particles, consistent with the chemical content of diatoms.

Figure 7 shows what is probably a biological fragment, since the elemental peaks are very low, and there are a few silicon-containing particles in the background. In addition a cellular structure is visible.

Figure 8 shows particles with crystalline structure. The spectrum shows several elemental peaks, calcium and silicon being the strongest. The dot maps indicate that the crystals are a calcium compound, possibly the insoluble calcium salt of an organic acid such as oxalate, citrate or aconitate. There are silicon containing particles in the background which are not part of the crystalline structures.

Figure 9 shows a large particle with a spectrum very strong in aluminum, but with a silicon peak as well. Dot maps indicate that the particle is aluminum, and that the silicon is present in two separate particles.

Figure 10 illustrates a particle having three major peaks, silicon, calcium, and phosphorus. X-ray maps indicate that the particle contains all three elements, and that there are additional small particles containing silicon in the background.

These illustrations show the power of the scanning electron microscope-energy dispersive elemental analysis system to help in the identification of unknown particles. Additional electron micrographs are discussed elsewhere in these Proceedings by Ingber *et al.*

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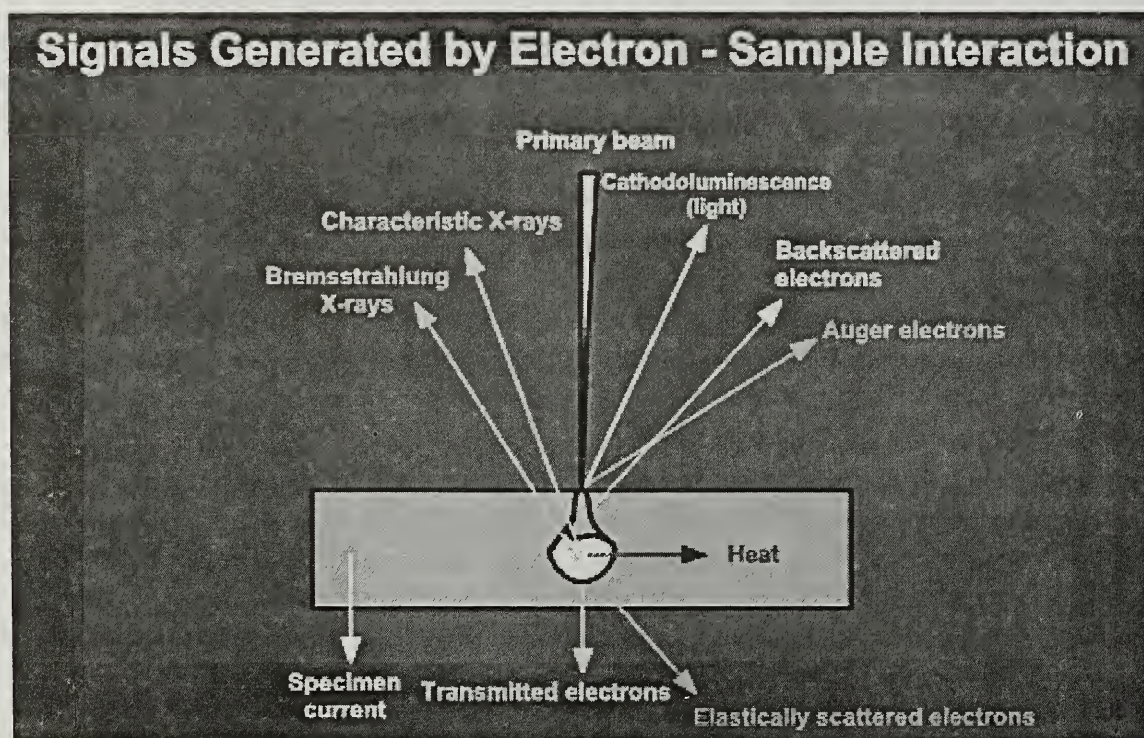


Figure 1. Signals generated by electron-sample interaction.

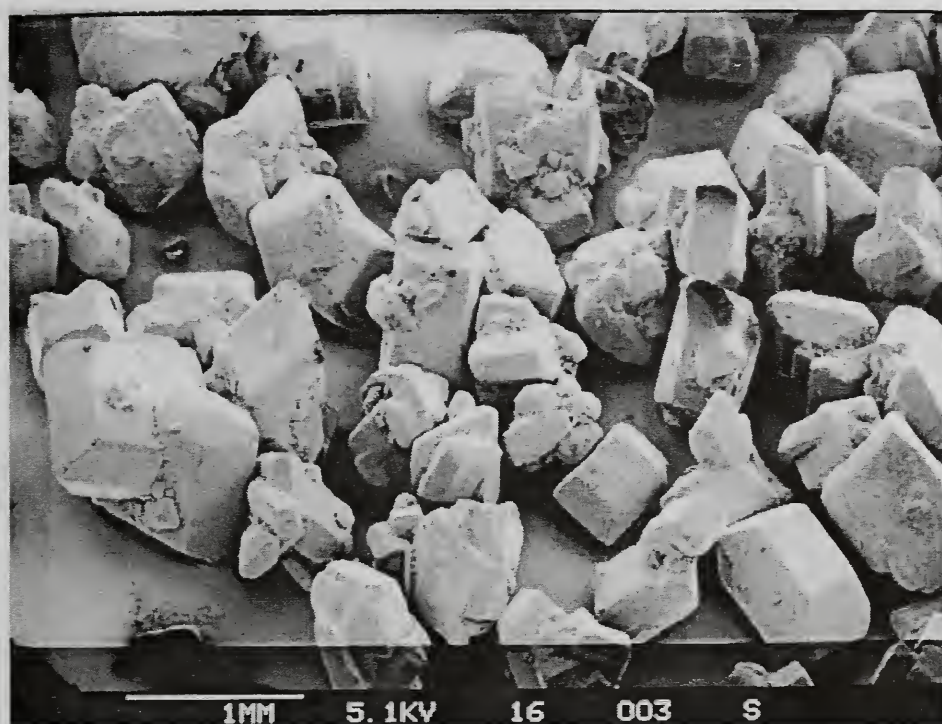


Figure 2. Refined sugar crystals.



Figure 3. Turbinado sugar crystals.

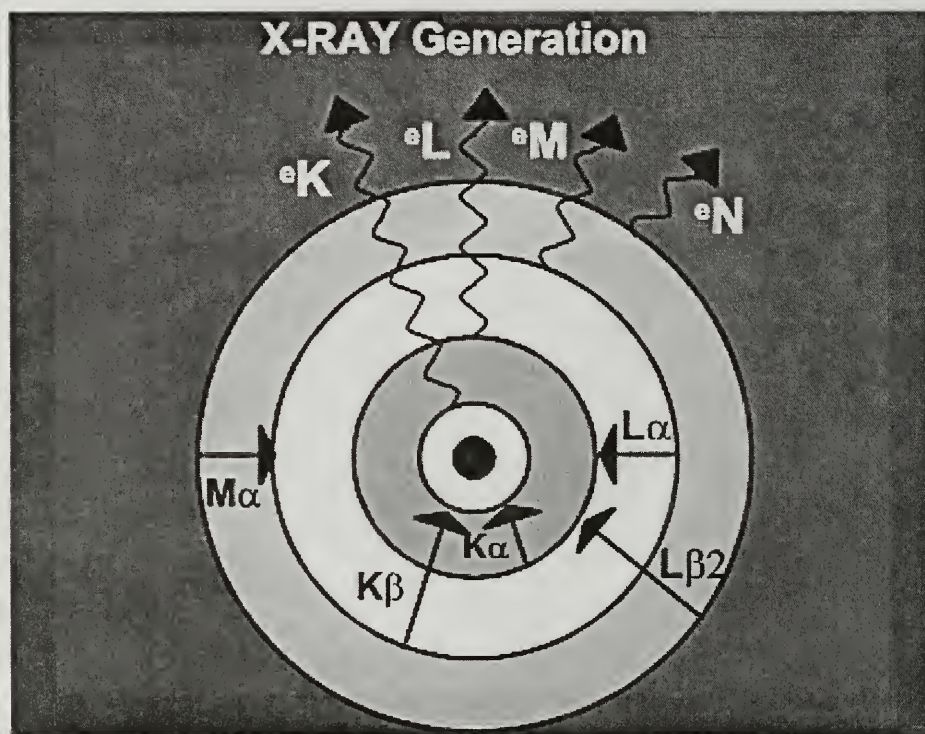


Figure 4. Generation of x-rays by rearrangement of atomic electrons.



Figure 5. A typical x-ray energy spectrum.

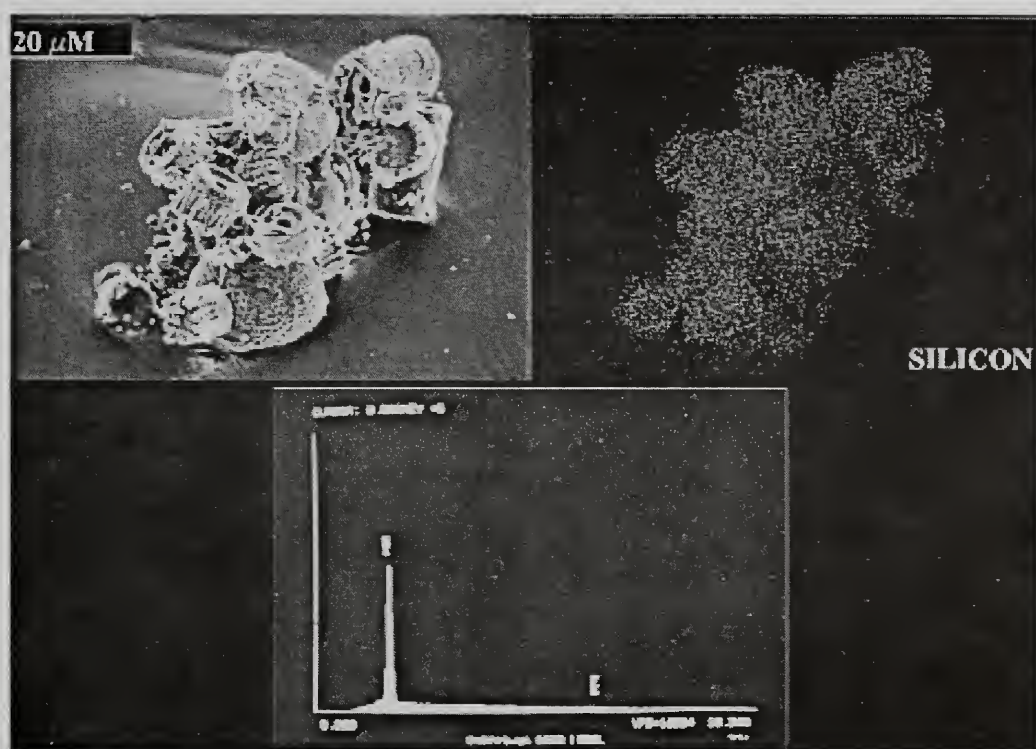


Figure 6. Group of diatoms with silicon dot map and spectrum.

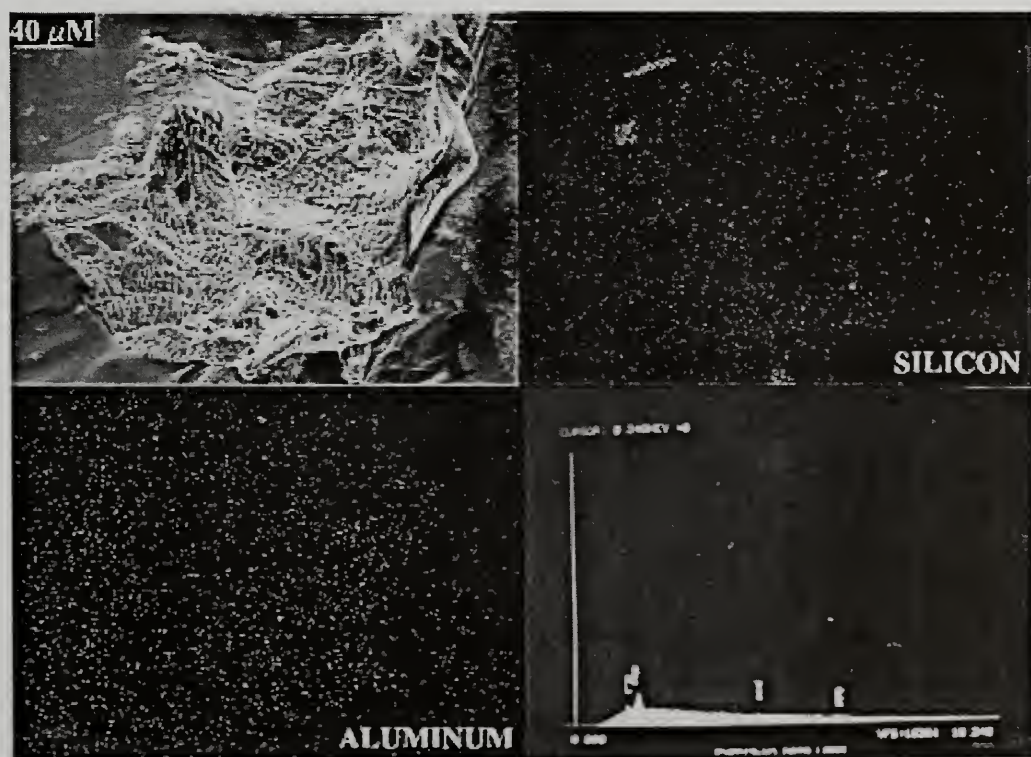


Figure 7. Particle (probably biological) with silicon particles in background. Note cellular structure.

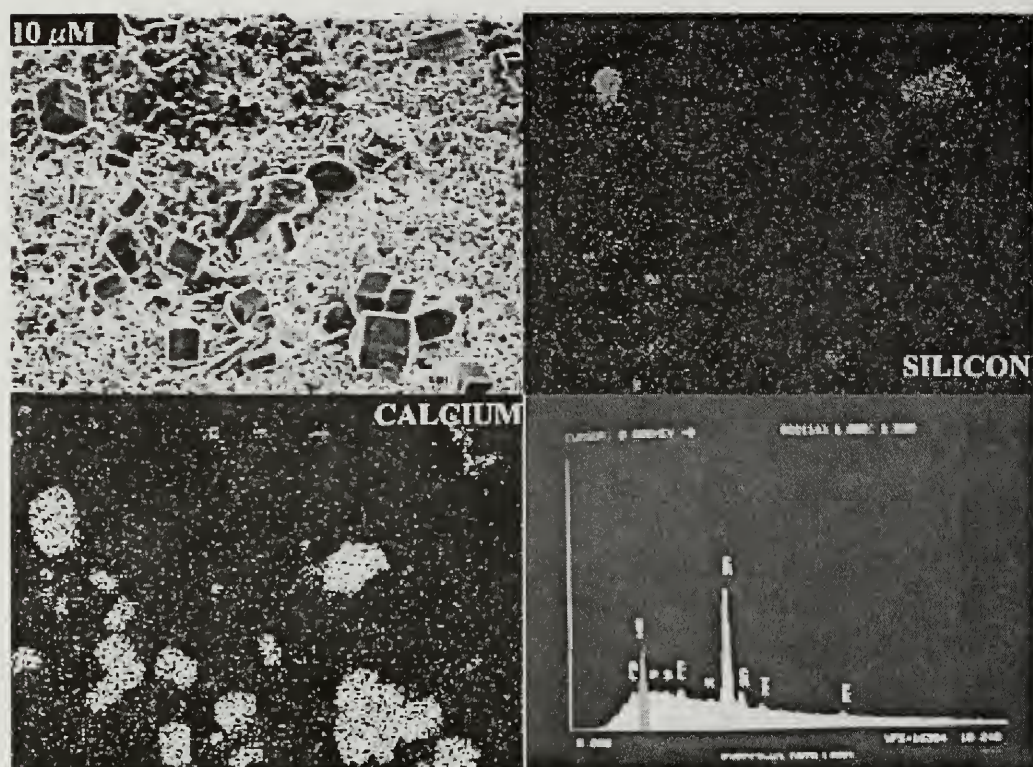


Figure 8. Field with crystals, and spectrum showing strong silicon and calcium peaks. Calcium is mapped at crystal locations and silicon is not. These are possibly insoluble calcium salts of an organic acid, such as oxalate, citrate or aconitate.

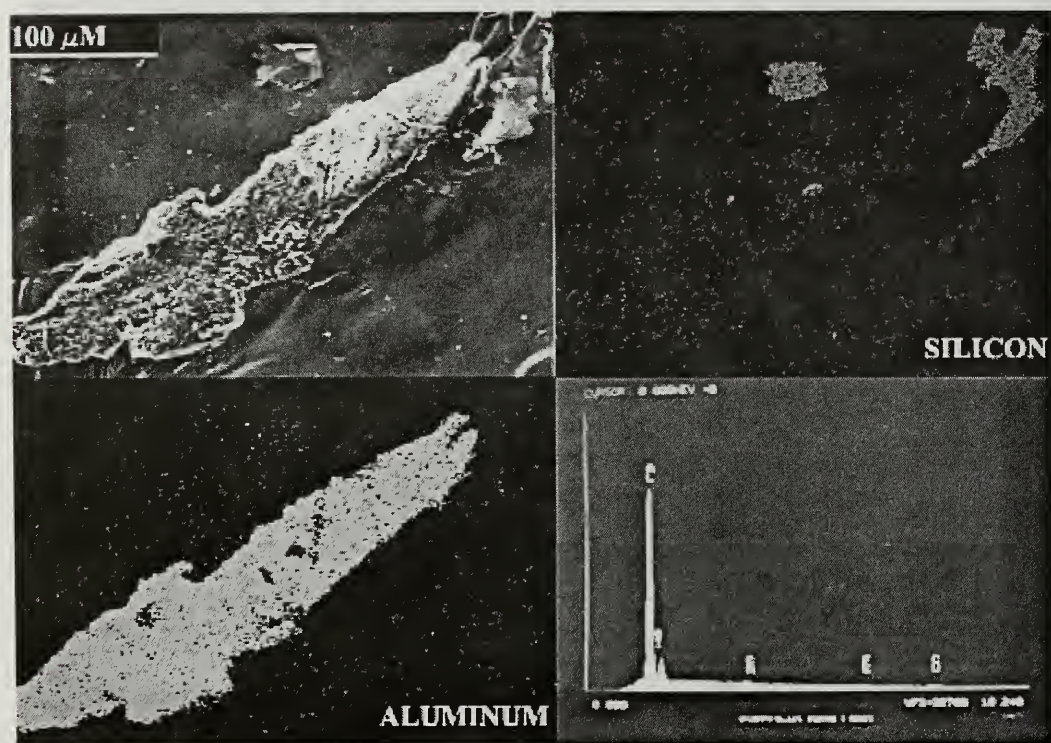


Figure 9. An aluminum particle with small silicon particles.

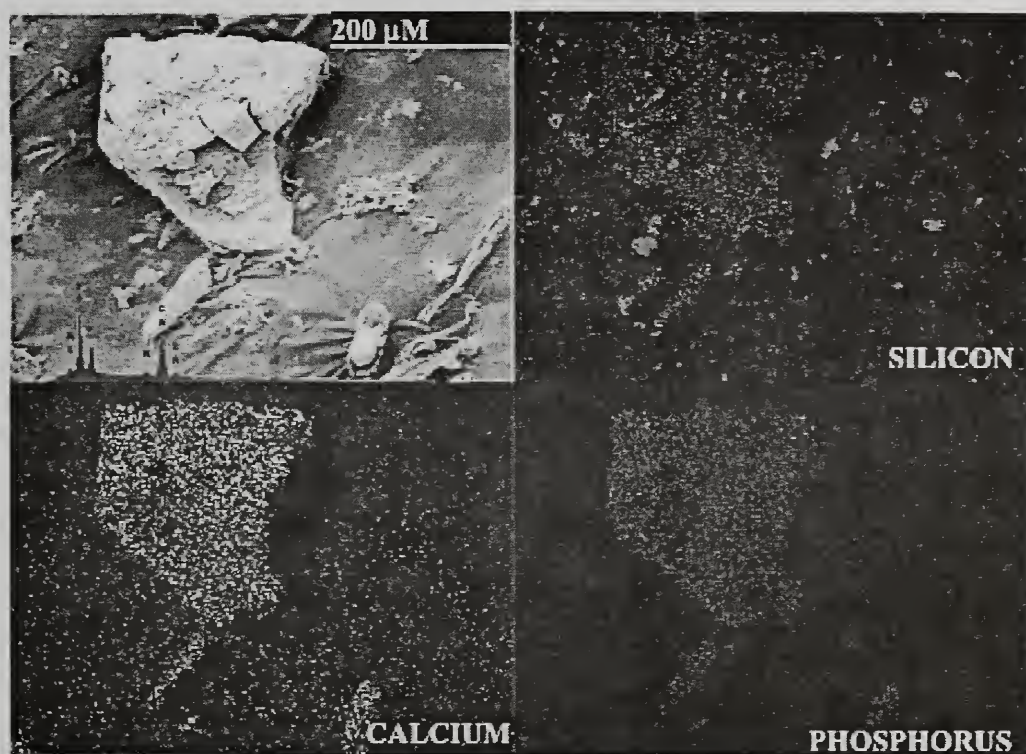


Figure 10. Particle containing silicon, phosphorus and calcium.

DISCUSSION

Question: Do you intend to apply any of these techniques to acid beverage floc?

Blanco: We have not done that yet. As you know, most floc is very difficult to isolate as it breaks up with a small amount of motion.

DETERMINATION OF SOLIDS IN MOLASSES BY DRYING METHODS

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ABSTRACT

Vacuum oven dry weights of molasses and syrups have been used as a measure of concentration and mass balance for many years as a confirmation of the accuracy of Brix refractometric measurements. The differences in solids between determination by refractive index and drying are often a few percentage points lower in the residue weight after vacuum drying than found by refractometry. Just the opposite might be expected since the greater error often found on drying substances is the inability to remove all the water during the treatment with heat and vacuum. In this work °Brix was compared with solids estimated by first drying a thin film (about 1 gram) of the molasses under ~0.1 mm Hg at 50° C overnight, followed by drying at 90° C to constant weight. The dried molasses or syrup did not show appreciable sucrose degradation with IC determination of sugars, although apparent increase in sucrose in some samples can be attributed to co-elution of colorant formed on heating. Invert sugar decreased during drying presumably through degradation reactions. Insoluble aggregated polymeric material formed under heating was filterable. Gel permeation chromatography of the filtrate also did not reveal any new soluble polymer development in the solid cake as a result of the drying conditions. Gel permeation chromatography also did not reveal any new polymer development in the solid cake as a result of the drying conditions. Thin film drying of molasses and syrups under heated vacuum is a reliable check of refractometric solids.

INTRODUCTION

The International Commission for Uniform Methods of Sugar Analysis (ICUMSA) ratified at the 21st Session in 1994 (6) that moisture is considered present in sugar crystals in three forms:

- Free moisture, or that contained on the surface of the crystal coming from the centrifugals which is easily and quickly removed on drying.
- Bound moisture, or that contained in the glassy layer on the surface and in the re-entrant angles which is only released slowly as the glass crystallizes.
- Inherent moisture, or that included within the crystal structure and only released in general by grinding.

In Method GS2/1/3-15 (6) the determination of free moisture in sugar crystals is carried out in a forced air drying oven at atmospheric pressure.

Although these moisture categories can be defined for sugar crystals, molasses is a very complex, almost glassy substance. Dry substance determination in molasses is recommended by the vacuum oven drying method for molasses on sand and is detailed in Method GS4/7-11 (6). Previously, "water" or "moisture" were not recommended to describe the volatiles in molasses because other organics or dissolved gases could also account for the total mass. Later, by use of chemical reactivity of water present, the Karl Fischer

moisture determination of molasses (6) demonstrated that reacted water exceeded by 1% weight losses as total heat volatiles evaporating from the molasses. In the absence of any method to determine "true dry substance", the residue after vacuum drying is "dry substance" and the volatiles "moisture". In the sand vacuum oven method for molasses, the maximum surface area of molasses is presented for evaporation while minimizing degradative reactions. In this way the least amount of occluded or bound water remains in the molasses dry substance.

ICUMSA also recommends for solids in molasses the determination of refractometric dry substance (RDS %) of molasses in Method GS4-13 (1994)(6). Apparent dry substance (°Brix) of molasses is described by ICUMSA using a hydrometer (Method GS4-15)(6). Discussions of refractometric determination of solids in molasses are well detailed in the sugar literature such as the original Spencer and Meade *Cane Sugar Handbook* and successive editions to date (2). Comprehensive consideration of moisture in sugars was given by Goodrow (4) in his paper on the use of an infrared dryer in determining soft sugar moistures. Godshall and Miranda contributed much experimental data in their 1995 S.I.T. research paper (5). In this paper they consider the recent history of moisture testing by ICUMSA for raw sugar and white sugar. Actual determinations in their work were performed on white sugar, powdered sugar, and soft sugar using several devices now marketed for moisture determinations in addition to dynamic purge and trap in a heated inlet with inert gas sweep as well as ICUMSA oven drying Method GS2/1/2-15(6). The ICUMSA oven drying method was used as the comparison for sugar samples determined by the other methods described by Godshall and Miranda (5). Method GS2/1/2-15(6), drying at atmospheric pressure and a temperature of 105°C, employs 20 to 30 g of sample for a heating time of three hours.

Most recently, Vaccari and coworkers (11) reported collaborative tests for ICUMSA for moisture in cane and beet molasses as well as cane raw sugar using updated reagents for the modern Karl Fischer moisture titration. Statistical evaluation of the Karl Fischer moisture determination as compared to the oven drying method for the above samples of molasses indicated that the Karl Fischer moisture is higher than the oven moisture by more than several percent in every case (11). Variability in raw sugar moisture by the Karl Fischer method ruled out its adoption as an official ICUMSA procedure at present. The modern Karl Fischer moisture determination in the collaborative study of Vaccari, Godshall, and Nemeth (11) should be acceptable as Official ICUMSA method for the analysis of both cane and beet molasses.

OBJECTIVES

In the present study on moisture in molasses the following objectives were set as goals:

To explore the use of thin-film drying of molasses under heated high vacuum with a view to discern whether sugar degradation is an integral part of weight loss during moisture removal by using ion chromatography with electrochemical detection to monitor sugar concentrations and gel permeation chromatography to determine polymer formation.

To evaluate refractometer Brix measurements for dissolved solids in molasses by comparison to vacuum oven dehydration of molasses using an alternative dynamic purge and trap methodology to remove moisture in an inert helium atmosphere.

EXPERIMENTAL

Sugarcane final C molasses samples were collected from several commercial sources during 1995-1997. A sample of sugarbeet ion exclusion separator fraction rich in sucrose was taken in the 1996 processing period and labeled "Extract".

Analytical methods were from the International Commission for Uniform Methods of Sugar Analysis *Methods Book*, April, 1994 (6), and Methods of the Association of Official Analytical Chemists, 16th edition (3). Analyses performed by ICUMSA methods included refractometer Brix, color, and dry substance solids by heated vacuum oven drying.

Vacuum oven drying of molasses was carried out in a VWR Model 1845 electronically controlled vacuum oven. The vacuum pump was a Welch DirecTorr Model 8834 rotary oil vacuum pump and cold finger traps in line were held at -40°C with a Neslab immersion cooler. The molasses sample (about 1 gram) was spread in a thin film on the bottom or sides of a tared 100 ml tall form beaker prior to final weighing to 0.1 mg. The samples were dried for 14 hours at 0.1 mm pressure at 50°C , the oven brought to atmospheric pressure with a calcium sulfate drying tube on the inlet, the foam carefully pulverized with a 10 mm glass stirring rod, and the beakers returned to the oven for another 10 hours at 90° . The final samples were cooled in a desiccator over anhydrous calcium sulfate before weighing. A final weighing was made after this last 10 hour period to ensure that constant weight has been achieved.

The molasses moisture was also determined in a heated closed inlet, purge and trap device with helium sweep. The apparatus was described by Legendre and Fisher (8) and U.S. Patent #4,245,494(9). Into a 9 x 89 mm glass cartridge containing about 0.5 gram of fine glass wool (Corning #3950), the molasses sample (about 100 mg) was spread in a thin film and weighed to the closest 1 milligram. The cartridge was placed in the external closed inlet device at 130°C at 40 ml/min helium carrier flow with effluent vented to the atmosphere for 25 minutes. The cartridge was weighed after purging to estimate moisture loss.

High performance liquid chromatography of sucrose, glucose, and fructose in the molasses samples was performed on a Dionex PAC-1 column with pulsed amperometric detection using the Dionex AI-450 integrating computer program (1,12). The column was run at ambient temperature with 100 mM carbonate free sodium hydroxide in purified water (18 megohm-cm resistivity) and flow rate of 1.0 ml/min with a 200 mM sodium hydroxide wash at the end of the run and re-equilibration to 100 mM eluent before the next run. Samples were filtered through a $0.45\ \mu\text{M}$ nylon filter prior to injection. Calibration responses with 2-amino-2-deoxy-glucose internal standard were run repeatedly over the entire period of sampling and membrane separation experiments.

Gel permeation chromatography for colorant polymers (7, 10, 12) was carried out using a Shimadzu high performance liquid chromatography system equipped with an SPD-6AV ultraviolet detector and RID-6A refractive index detector using the Shimadzu LC-10AD pump, an SPD-6AV ultraviolet-visible detector, an RID-6A refractive index detector, and Rheodyne 7125 injection port. A $100\ \mu\text{l}$ injection sample loop was employed for samples filtered through $0.2\ \mu\text{l}$ filter prior to injection. Three TSK-GEL PW-XL columns in order as G6000, G5000, and G4000, corresponding to decreasing pore sizes, with appropriate TSK guard column. Samples were uniformly diluted to 15° Brix with elution solvent prior to filtration. Solvent was 0.1 M sodium chloride in purified water (18 megohm-cm resistivity) that was modified with 10 % HPLC grade

acetonitrile. Flow rate was 0.6 ml/min. The ultraviolet detector was set at 210 nm. Calibration standards were purchased from Scientific Polymer Products, Inc., New York, NY. These standards are sodium polystyrene sulfonates of molecular ranges from 4000 Da to 1.2 million Da. Quantitation of the peaks was calculated from the absorbance of the standards. A Dionex AI-450 laboratory data analysis system was used to integrate peaks as well as to sample peak area slices for polymeric calibration curves and molecular weight determinations.

RESULTS AND DISCUSSION

Choice of six typical market molasses samples was made on the basis of seeing whether the present thin film vacuum oven drying method was affected by kinds of polymers present in the molasses as byproducts of processing. For instance, in many of the foreign molasses samples used it is known that sulfitation is part of the processing, which leads to new polymeric species. In Table 1 comparison of °Brix for each sample with dry substance and moisture loss indicates that more moisture was lost in the typical molasses sample than the refractometric solids would indicate. These differences can range up to several percent. More than a thousand molasses samples have been determined in this laboratory by the thin film vacuum oven drying method with similar results: more moisture is removed and less dry substance solids are found to be present than the refractometric solids method would indicate. In terms of possible moisture entrapment in the molasses foam produced, just the opposite result would be expected.

The beet sugar ion exclusion extract (Sample 5) differed somewhat from the other samples in the refractometry solids in that the dry substance was almost the same. On the other hand, sugarbeet molasses extract is a highly purified material and has different physical properties than the sugarcane molasses in the other samples. When compared to moisture removal by the dynamic purge method, the thin film vacuum oven drying method has only a slight difference of a few tenths of a percent less solids than the purge drying. As reported earlier by Godshall and Miranda for sugars (5), the dynamic purge method gave results quite similar to the ICUMSA oven drying method. The question then remains: Why do both of the reproducible drying methods reported in Table 1 differ from °Brix refractometer solids for these samples?

Figures 1 and 2 show the rate of change for moisture loss during vacuum drying for Venezuela and Nepal molasses. The samples were taken to constant weight even though the process took more than 24 hours because it was important to find the limit of dry substances after total moisture removal. Upon linear regression analysis the major weighing points (as averages of two determinations) follow essentially a straight line relationship ($r^2 = >0.9$). This was interpreted as meaning that there was no unusual entrapment of moisture in the polymeric matrix.

Another question which comes up immediately is whether there is destruction of components causing the loss of dry substance mass as compared to the refractometry solids. In Table 2 a comparison of ICU color at pH 7 of molasses samples before and after oven drying indicates that there is only a small change in total ICU color after heating. These facts emphasize that only slight additional browning reactions have occurred. Interestingly enough, the same is true in the dynamic purge desorber even though the temperature is 130° C for 25 minutes. Presumably, the inert helium gas prevents oxidative polymerization of the molasses solids. The Venezuelan and Mexican molasses already had high color values. By comparison, the sugarbeet

molasses extract was quite low in color from the efficient ion exclusion separator. The extract did not gain a great deal of color, however, on heating in vacuum.

To determine the effect of vacuum drying on invert sugar and sucrose concentration, ion chromatography of the sugars was compared to °Brix and dry weight solids in the undried molasses samples are shown in Table 3. Although not listed, the pol values for each molasses are close to the percent sucrose. There is considerable invert sugar in the Venezuelan and Mexican samples, a fact known when these molasses samples were selected for the study because of the potentially high reactivity of these reducing monomers. In Table 4 ion chromatography of the dried substance for the samples in Table 3 is listed for invert sugars and sucrose. During drying polymers congealed or aggregated, which resulted in turbid solutions. Before ion chromatography the reconstituted samples were filtered through a 0.2 μ M nylon membrane. In Table 4 invert sugar is lower after drying than in Table 3. The loss of invert in the molasses samples can be explained by reaction with polymers to form higher molecular weight insoluble aggregates. The sucrose level apparently increases because some of the colorant molecules formed also co-elute with sucrose. Overall the values for sugar concentrations are similar with understandable variations depending on reactive components in the individual processing streams.

Similarly, in Table 5 gel permeation chromatography of a Nepal molasses sample before and after drying is compared for ultraviolet detector response in major integrator peak slices as colorant absorbance quantitation. No detectable difference in colorant generation is shown to have occurred during the heating and vacuum drying phase of dry substance estimation. In Table 6 all five of the molasses samples are similarly compared for colorant formation as estimated by gel permeation chromatography. The mass balance indicated no deterioration in any of these diverse molasses samples due to thermal degradation processes.

SUMMARY AND CONCLUSIONS

Vacuum oven dry weights of molasses and syrups used as a measure of concentration and mass balance generally gave total solids several percent less than Brix refractometric measurements.

Thin-film drying of molasses under heated high vacuum did not effect sugar degradation as an integral part of weight loss during moisture removal when determined by ion chromatography with electrochemical detection to monitor sugar concentrations and gel permeation chromatography to determine polymer formation.

Dynamic purge and trap drying of molasses in a heated inlet with inert gas sweep is a very efficient and quick method (25 minutes) to determine moisture which gave results slightly higher than vacuum oven drying but consistently similar. Results were also several percentage points less than refractometer °Brix on molasses.

Thin film drying of molasses and syrups under heated vacuum is a reliable measure of moisture in molasses.

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Table 1. Study to determine sugar decomposition on vacuum over drying.

Samples of molasses representative of sugar industry	°Brix	Percent vacuum oven dry weight solids (%)	Average vacuum oven dry weight solids (%)	Percent dry weight solids (%) Purge and trap 130°C for 25 minutes @ 40ml/minHe	Average percent dry weight solids (%) Purge and trap 130°C for 25 minutes @ 40ml/minHe	% difference Purge and trap 130°C and vacuum oven drying	Percent deviation from °Brix value
1A. Louisiana A 10-6-95	80.2	78.34	78.40	78.49	78.55	0.15	-2.05
1B. Louisiana A 10-6-95	80.2	78.47		78.62			
2A. Nepal 4-28-97	76.64	72.17	72.10	72.31	72.23	0.14	-5.75
2B. Nepal 4-28-97	76.64	72.02		72.16			
3A. Mexico 8-5-97	80.2	74.29	74.02	74.43	74.16	0.14	-7.53
3B. Mexico 8-5-97	80.2	73.74		73.88			
4A. Venezuela 5-9-97	81.49	75.25	75.22	75.39	75.36	0.14	-7.52
4B. Venezuela 5-9-97	81.49	75.19		75.34			
5A. Beet extract 11-18-96	68.3	68.39	68.32	68.52	68.45	0.13	0.22
5B. Beet extract 11-18-96	68.3	68.25		68.38			
6A. Venezuela 6-17-97	80.3	77.00	76.97	77.15	77.12	0.15	-3.96
6B. Venezuela 6-17-97	80.3	76.94		77.09			
Average standard deviation (+/-)	77.86 4.53		74.17 3.35		74.31 3.31	0.14 0.05	-4.55

Table 2. Comparison of ICU color at pH 7 of molasses samples before and after oven drying.

Sample	Initial °Brix	Wet weight sample (g)	Sample diluted 1->100 °Brix	Absorbance sample dil. 1->2	Color ICU (X0.001)
1A. Louisiana A 10-6-95	80.2	1.1458	0.9189	0.188	41
1B. Louisiana A 10-6-95 (dried)	80.2	1.0024	0.8039	0.1575	39
2A. Nepal 4-28-97	76.64	1.0862	0.8325	0.3675	88
2B. Nepal 4-28-97 (dried)	76.64	1.1738	0.8996	0.361	80
3A. Mexico 8-5-97	80.2	1.1078	0.8885	0.6515	147
3B. Mexico 8-5-97 (dried)	80.2	1.0497	0.8419	0.6505	155
4A. Venezuela 5-9-97	81.49	1.1224	0.9146	0.6805	149
4B. Venezuela 5-9-97 (dried)	81.49	1.0924	0.8902	0.7575	170
5A. Beet extract 11-18-96	68.3	1.1306	0.7722	0.029	8
5B. Beet extract 11-18-96 (dried)	68.3	1.0366	0.7080	0.046	13
6A. Venezuela 6-17-97	80.3	1.1632	0.9340	0.614	131
6B. Venezuela 6-17-97 (dried)	80.3	1.0171	0.8167	0.5025	123

Table 3. Vacuum drying study comparing °Brix with dry weight solids. Undried samples.

Sample name	Average percent glucose	Average percent fructose	Average percent invert sugar	Average percent sucrose	Average percent total sugars	°Brix-tc molasses and syrups	Average vacuum oven dry weight solids (%)
Louisiana B	5.81	5.02	10.83	57.12	67.95	77.92	75.40
Nepal	5.77	7.73	13.49	45.00	58.49	76.64	72.10
Mexico	13.30	13.09	26.39	41.62	68.01	80.2	74.02
Venezuela 5-9	9.38	9.51	18.89	40.16	59.05	81.49	75.22
Beet extract	1.11	0.68	1.78	65.63	67.41	68.3	68.32
Venezuela 6-19	10.83	9.44	20.28	34.30	54.57	80.3	76.97

Table 4. Vacuum drying study comparing °Brix with dry weight solids. Samples dried at 90°C in vacuum to constant weight.

Sample name	Average percent glucose	Average percent fructose	Average percent invert sugar	Average percent sucrose	Average percent total sugars	°Brix-tc molasses and syrups	Average vacuum oven dry weight solids (%)
Louisiana B	3.456	4.677	8.133	56.284	64.417	77.92	75.40
Nepal	3.391	5.607	8.998	53.256	62.254	76.64	72.10
Mexico	8.220	9.920	18.141	52.697	70.838	80.2	74.02
Venezuela 5-9	5.559	7.357	12.916	50.056	62.972	81.49	75.22
Beet extract	0.551	0.432	0.983	66.949	67.932	68.3	68.32
Venezuela 6-19	6.737	7.417	14.153	46.320	60.473	80.3	76.97

Table 5. Gel permeation chromatography of molasses drying samples to compare molecular weight distribution of solids for undried molasses with vacuum oven dry weight solids.

2A. Nepal 4-28-97 °Brix 76.64		2B. Nepal 4-28-97 (dried) °Brix 76.64	
Ret time	Concentration μG/mL	Ret time	Concentration μG/mL
31.21	3.38	31.49	3.55
44.03	5.28	44.1	5.53
55.52	419.91	56.09	720.09
58.06	2417.83	56.63	2230.64
60.14	294.92	60.31	30.135
63.46	218.77	63.53	212.58
64.59	90.22	66.03	108.08
66.21	115.55	66.62	152.11
69.45	51.17		
75.07	55.03	75.1	72.10
	3672.07 PPM colorant 24.56%		3806.03 PPM colorant 24.85%

Table 6. Effect of drying on colorant polymer formation for molasses vs vacuum oven dehydrated solid gel permeation chromatography with ultraviolet detection.

Molasses sample	°Brix	Average vacuum oven dry weight solids (%)	Molasses as received % colorant	Molasses after drying % colorant
Nepal 4-28-97	76.64	72.10	24.56	24.85
Mexico 8-5-97	80.2	74.02	22.02	22.36
Venezuela 5-9-97	81.5	75.22	22.16	23.70
Beet extract 11-18-96	68.3	68.32	3.61	3.80
Venezuela 6-17-97	80.3	76.97	24.64	24.52

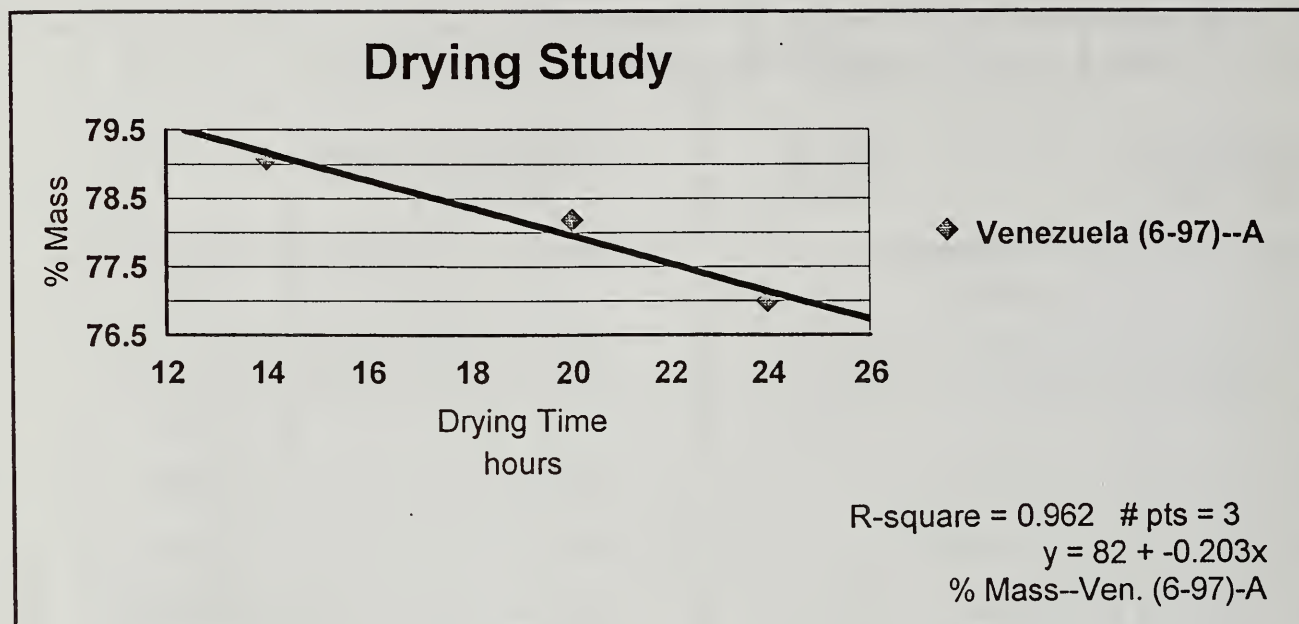


Figure 1. Rate of change for moisture loss during vacuum drying. Venezuelan molasses.

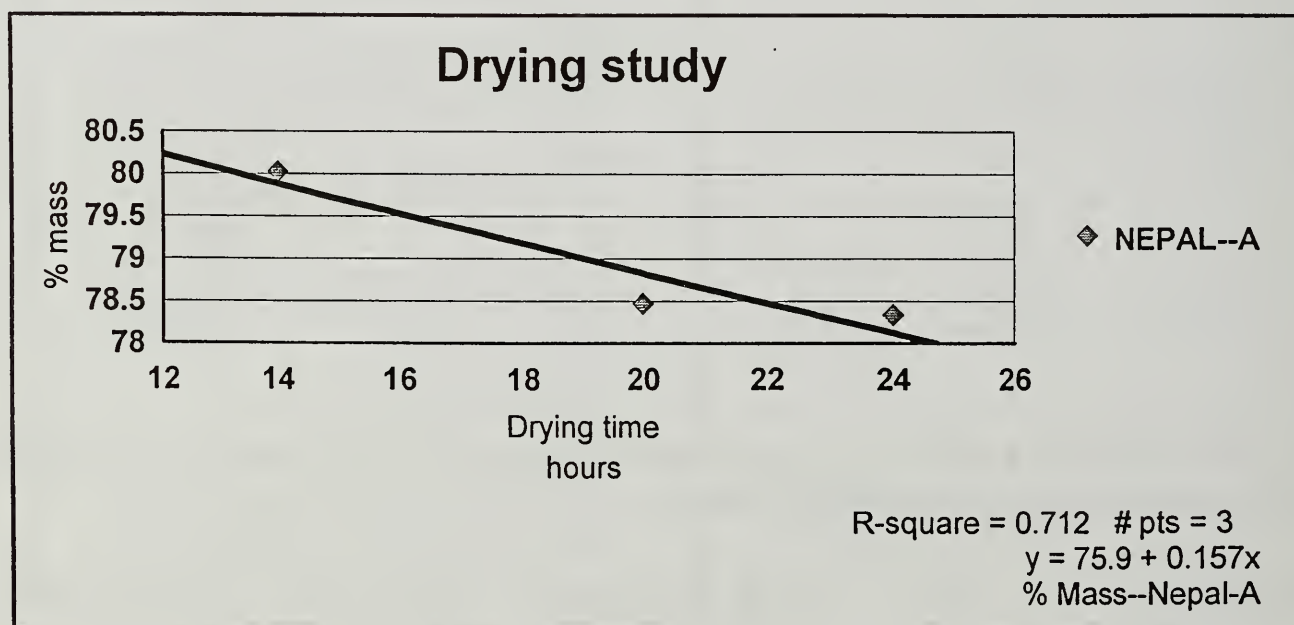


Figure 2. Rate of change for moisture loss during vacuum drying. Nepal molasses.

DISCUSSION

Question: Prior to the refractometric determination of Brix, do you dilute the molasses samples?

Vercellotti: Generally, the samples were 70-76 Bx except the extract sample which was about 67 Bx. The analytical Brix was done in the usual way with dilution. Checking the syrups used for drying was done by running a refractive index on the undiluted syrups.

Question: Do you think it would be of any value to determine the water content of the molasses instead of trying to determine the solids? Perhaps by a Karl Fischer method?

Vercellotti: We have tried Karl Fischer on a number of occasions and we had very divergent results. We are not really satisfied with the precision of our results. We get much better precision by doing the dry substance solids.

I know these are important questions within ICUMSA, and we have who is probably the world's expert on molasses drying here, Dr. Vaccari. I hope that a solution will be found as to what the "touchstone" should be, whether it is moisture or dry substance solids.

Question: How sure are you that during the oven drying, there is not evaporation of volatile components from the molasses?

Vercellotti: We have analyzed these samples by using the same purge-and-trap desorber that is used on the gas chromatograph. We put all of the organics and the moisture onto the same GC column. We then determine the area response from the flame ionization detector as compared to a known quantitative standard for general organics, and we find that the general organics are only about 0.1-0.2%, and the rest is assumed to be moisture. Gilbert Spencer, back in 1921, also asked questions about organic volatiles. We have done very many of those samples.

Comment: May I also point out before the next question that Dr. Vercellotti's GC method is a moisture determination method, and a rather more reliable one than Karl Fischer, in my experience.

Question: Did you run any hydrometer Brix to see how it correlated to the drying Brix?

Vercellotti: No, we did not.

Comment: For the next session of ICUMSA, we will propose an official method for the analysis of water in molasses using Karl Fischer methodology. In our experience, when we compare the three methods - Karl Fischer (which determines the exact amount of water), drying matter, and the Brix, we obtain a higher level of water using Karl Fischer, a lower amount using oven drying, and a much lower amount of water using Brix. These differences are higher than the content of nonsugar. In the oven drying method, you cannot eliminate all of the water. The amount of water that remains depends on the presence of nonsugars, which bind part of the water. The Brix is an apparent measure because the refractometric apparatus is calibrated using pure sucrose. As the level of nonsucrose compounds increases, the Brix method becomes less and less reliable, and is not a "true" measure. In the analysis of Quentin molasses, for example, the error on dry substance obtained by Brix is higher and higher as you increase the exchange of sodium and potassium with magnesium, because magnesium crystallizes water and this water cannot be determined using refractometric measurement.

Vercellotti: Thank you, Dr. Vaccari. This is just this kind of discussion I had hoped to stimulate at this meeting.

ACID BEVERAGE FLOC FROM BEET SUGARS

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ABSTRACT

Acid beverage floc (ABF), a flocculated turbid material that can form in sugar sweetened, acidified, carbonated beverages after several days standing, is a customer problem to beverage bottlers and their suppliers of sugar. ABF from beet sugar has been reported (Eis *et al.*, 1952; van der Poel *et al.*, 1966) to be caused by a saponin from the beet plant. Recent work has shown the presence of several saponins in sugarbeet (Masiot *et al.*, 1994; Ridout *et al.*, 1994). Investigations at S.P.R.I. have confirmed that isolation and test procedures for saponins, as reported in the literature, are actually for oleanolic acid.

In this paper, observations on ABF and its causes are reported. ABF from beet sugar is proposed to have a two factor basis: a negatively charged component and a positively charged component interacting at acid beverage pH, to form a coacervate and subsequently coagulating into a floc. The negatively charged factor can be oleanolic acid, any of the saponins that contain a glucuronic acid moiety, or beet cell wall polysaccharide containing uronic acids. The positively charged component can be protein or peptide, with isoelectric point above the beverage pH of 2.5-3.0. ABF can be made readily by adding these components to non-floccing sugars.

Acid beverage floc and its causative factors can be removed by intense or tight filtration. The negatively charged components can be removed to a great degree by formation of their calcium complexes (at pH above 8.5) and subsequent filtration.

INTRODUCTION

Acid beverage floc

Acid beverage floc (ABF), which can form in sugar-sweetened carbonated soft drinks after several days standing, has been ascribed to both beet and cane sugars. In general, any haze or turbidity in a soft drink is referred to as "floc", but there are specific characteristics which define acid beverage floc, most notably that it disappears upon shaking. Beet and cane flocs can appear as turbidity or as "cotton ball floc". Beet sugar floc can be more granular in appearance and less fluffy than cane sugar floc, in our experience. Figure 1 shows a typical floc from beet sugar (sample on the right), and a sample beverage made from non-floccing beet sugar (sample on the left). Beet sugar floc has, for many years, been ascribed to saponins (Eis *et al.*, 1952; van der Poel *et al.*, 1966; Carruthers *et al.*, 1967), but in our tests, the addition of isolated saponin added at levels resembling those in sugar to trial sugar floc tests do not necessarily produce floc. Eis *et al.*, (1952) stated that "separated floc" (by which was meant all material precipitable at pH 2.0) can "produce effervescence and flocculation when sufficient neutral solution of the floc is added to carbonated beverages". In the authors' experience, "sufficient" is far above the levels of saponin reported in white sugars (<1 to 30

ppm). "Sufficient" levels are above several hundred ppm. It is therefore of interest to isolate sugarbeet saponins for further study of their effect on acid beverage floc formation. The evidence for the responsibility of sugarbeet saponins for this phenomenon may be circumstantial.

Acid beverage floc from cane sugars has been found, in the general case, to have two causative factors: a polysaccharide with glucuronic acid groups and a protein. (There is at least one other floc, a specific regional acid beverage floc, caused by specific regional infection.) In the general case, the polysaccharide is derived from plant cell wall material. The protein may come from the cane plant, or may be residual from enzyme addition. The glucuronic acid and the primary amine residues become oppositely charged at beverage pH, and, through charge attraction, come together to form a coacervate as the basis for a floc network. Suspended solids in the solutions (e.g. colloidal material) and high molecular weight solubles (e.g. dextran, starch) can come out of solution and enhance the appearance of a floc that has already formed (Clarke *et al.*, 1977, 1980).

Saponins

Saponins are a class of compounds widely distributed in the plant kingdom in legumes, roots, shrubs and bushes, in varying degrees of concentration (Waller and Yamasaki, 1996). Various saponins have been used as soaps because of their surface active properties - hence their name. Saponin-containing plants, or their extracts, have been used in herbal medicine, in treatment of various complaints including liver and cholesterol related diseases (Ireland *et al.*, 1986), and as anti-fungal agents (Hallanoro *et al.*, 1990; Waller and Yamasaki, 1996).

Saponins fall into two classes: triterpene-based and steroid-based. To the base aglycone are attached sugar group(s) and glucuronic acid, which define the molecule as a saponin.

Sugarbeet (*Beta vulgaris*) is known to contain at least three triterpene-based saponin structures, all glucuronic acid glycosides of oleanolic acid (Ridout *et al.*, 1994), as shown in Figure 2.

Two additional compounds, seco-glycosides of saponins, isolated from beet leaves and roots, have recently been identified (Massiot *et al.*, 1994). Up to six beet saponins have been postulated.

Saponins are reported to be found in sugarbeet at levels of 0.01% to 0.2% of beet (Carruthers *et al.*, 1961; van der Poel *et al.*, 1966; Hallanoro *et al.*, 1990; Schiweck *et al.*, 1991), and at less than 100 ppm, generally less than 20 ppm, in white sugar. Saponins are most densely concentrated just under the sugarbeet skin, where they function as plant defense compounds, against disease and against frost damage, and are located in cell membranes (Hallanoro, *et al.* 1990). They are most highly concentrated in small beets grown in warm climates.

Work by the current authors (Roberts *et al.*, 1996) comparing isolation systems and their products indicates that material reported as "saponin" in sugars and process streams may in fact be only oleanolic acid. However, since oleanolic acid is derived from saponins, their presence was still indicated. Recent work (Roberts *et al.*, 1997) has shown that oleanolic acid plus a molecule that is positively charged at beverage pH, probably a protein or peptide, are the factors that cause acid beverage floc from beet sugars. Saponin(s),

are hydrolyzed to oleanolic acid and substituent sugars at beverage pH. The authors proposed that the oleanolic acid moiety is the negatively charged factor in floc formation.

MATERIALS AND METHODS

EXTRACTION OF SAPONINS

Beet peelings, obtained from fresh sugarbeet in the S.P.R.I. labs, were subjected to several methods of extraction. The resulting extracts were compared by thin layer chromatography and by GC-MS.

1. Method of Rother, aqueous extraction (Rother, 1962).

Fresh beet peelings (5.5 kg) were covered with water in a blender and divided into small pieces. The slurry was heated to 90°, and filtered on fabric. The residue was suspended in water, heated, and filtered again on fabric. The pH of the filtrate was adjusted to 1.5 with HCl, heated to 90°C for one hour, and allowed to settle overnight. After settling, the supernatant liquid was decanted. The residue was mixed with filter aid and filtered; that residue was washed with water, adjusted to pH 1.5 with HCl, and allowed to air dry. The dried residue was extracted in a Soxhlet extractor with ethanol, the ethanol solution concentrated, and poured into water at pH 1.5. The precipitate was dissolved in hot ethanol and again precipitated by pouring into pH 1.5 water. The precipitate was filtered off on hardened paper, dissolved in water, and evaporated to dryness at low temperature; yield 3.0 g of brown material.

Analysis of this material by TLC (as described below) showed oleanolic acid (the aglycone, or sapogenin) and nothing corresponding to saponins. Mass spectroscopy analysis confirmed the presence of oleanolic acid. Apparently the harsh acidic treatment hydrolyzed the saponins, leaving only oleanolic acid in the isolation.

2. Method of Ridout et al, aqueous extraction (Ridout *et al.*, 1994).

In another experiment, 1734 g of beet peel was ground in a blender. The slurry was filtered on fabric and the residue mashed with water. The pH of the filtrate was adjusted to 1.5 with HCl, heated to 85°C for 15 minutes, cooled overnight, and filtered on fabric coated with filter aid. The filtrate was returned to the filter twice more and the residue was washed with warm 1N HCl. All filtrates were discarded. The filter was then washed with warm 2N NaOH solution until the filtrate was clear. The filtrate was placed in a large beaker and HCl was added to reduce the pH to 1.5. The precipitate was collected on fabric coated with filter aid as before, washed with 1N HCl, and the filtrate discarded. The filter was then washed with warm 2N NaOH. The filtrate was acidified to pH 1.5 with HCl, filtered through Whatman 542 paper, washed with water, and extracted with 500 ml of warm ethanol. The filtrate was evaporated to dryness, then taken up in water and freeze dried, yielding 2.0 g of brown material. TLC analysis showed oleanolic acid but no saponin.

3. Method of Ridout *et al.*, methanol extraction (Ridout *et al.*, 1994).

Freeze dried beet peel (650 g) was crumbled into small pieces and extracted in a Soxhlet extractor with methanol. The methanol was evaporated under reduced pressure, residue dissolved in water, and extracted several times with 1-butanol.

The butanol was evaporated and the residue was dissolved in water and dialyzed against flowing tap water in a 12,000 MW cut off bag for 24 hours. The material remaining in the bag was filtered, concentrated, and freeze dried. Yield, 5.6 g of cream colored material. This material was subjected to thin-layer chromatography and mass spectrometry, as below; it appeared to contain saponins.

Thin layer chromatography of isolates from aqueous extraction

Isolates prepared by the traditional aqueous extraction methods (1 and 2), with repeated extractions at pH 1.5 and washing with base, showed only oleanolic acid in the final dried extract, and no saponins. Oleanolic acid identification on thin layer chromatography (solvent system: chloroform; methanol; water 65:35:10), made visible by 2N H₂SO₄, or anisaldehyde spray, was confirmed by gas chromatography-mass spectrometry identification.

Method 1, of Rother (1962), using aqueous extraction and low pH, yielded 3g (0.05% on beet peel) brown solids; Method 2, of Ridout et al (1994), yielded 2 g (0.12% on beet peel), of brown material. Method 3 of Ridout et al (1994), similar to that of Ireland (1986) using methanol extraction and not including low pH treatment, yielded 5.6 g (0.8% on beet peel) of cream colored material. Thin layer chromatography of the methanol extracted material showed five major components, two of which traveled with an authentic saponin obtained from Sigma Chemical Co. It should be noted that "saponin extracts" supplied to S.P.R.I., Inc., by several sugar companies (sponsoring companies of S.P.R.I., Inc.) appeared to consist mainly of oleanolic acid.

Gas chromatography-mass spectrometry (GC-MS)

Samples containing oleanolic acid were converted to the trimethylsilyl derivative (TMS) using Pierce Tri-Sil[®] in pyridine solution. Gas chromatography (GC) was performed on a Hewlett Packard 5890. GC conditions were: 250°C for 10 min; increase temperature 5°C per min to 310°C for 10 min. The column was a 30 m x 0.25 mm fused silica with 0.25 μ film thickness of 5% phenyl methyl silicone. Oleanolic acid eluted at 21.11 minutes under these conditions. Mass spectrometry (MS) was conducted with a Hewlett Packard 5972 mass selective detector.

Charged species at beverage pH

Moving boundary electrophoresis on oleanolic acid was conducted in sucrose solution at pH3, adjusted with phosphoric acid. Oleanolic acid moved towards the anode. The oleanolic acid is thereby shown to have a negative charge at pH3 in sucrose solution (simulating acid carbonated beverage conditions).

RESULTS AND DISCUSSION

COMPOSITION AND STRUCTURE OF SAPONIN BEET ISOLATES

Saponins are known to exist in variety in any one plant - a single structure is not common. Variations in the sugar moiety structure and linkage position are observed. Sugarbeet saponins are no exception. The three forms shown in Figure 1 all have as their base unit oleanolic acid, a carboxylic acid triterpene.

It is evident from comparison of the aqueous methods of extraction with the methanolic method that the sugarbeet saponins are indeed present in the whole root, in the peel, just under the skin. It is also evident, from chromatographic and mass spectroscopic data, that the saponins extracted by aqueous methods have been hydrolyzed by the strong acid treatment so that only the aglycone (or sapogenin), oleanolic acid, remains.

This observation throws some doubt on earlier work, all of which isolated saponins by aqueous extraction with strong acid treatment. Were these earlier results the product of oleanolic acid only? Earlier workers did not have the benefits of GC-MS, but had to rely on colorimetric tests, which may give a false positive for saponins when oleanolic acid is present.

Recent work (Ridout *et al.*, 1994) found saponins by aqueous extraction, not in extracts of beet roots, but only in extracts from beet molasses, where the compounds may be expected to concentrate. Subsequent investigations (Massiot *et al.*, 1994) found saponins in methanol extracts of sugarbeet roots and leaves.

BEVERAGE FLOC

Floc tests (30%-50% rds, phosphoric acid to pH 2) were run on non-floccing sugars with the addition of varying amounts of beet extract, or commercial saponin (not from beet), or oleanolic acid. Saponin and oleanolic acid were also tried in combination with protein (gelatin and α -amylase were used). The methanolic extract of beet root formed a floc, as did the combination of oleanolic acid and protein.

The observation that saponins apparently are hydrolyzed during the acid extraction raises a basic question about reactions in and causes of floc formation. The assumption was that floc material was acid insoluble, and therefore the aqueous extraction method at low pH was developed. But do saponins themselves, if in white sugar and therefore in beverage, become hydrolyzed at beverage pH (about 2 - 2.5)? In that case oleanolic acid and not saponin would be responsible for floc formation.

In studies reported here, the only floc former (that is, floc former when used alone, without other reagent) that was isolated from sugarbeet was the methanolic extract, i.e., the extract that contains unhydrolyzed saponins. So it would appear that whole saponin - or at least whole when it enters the beverage - does form beverage floc. This methanolic extract contained many other compounds from beet in addition to saponins. Since the saponin is hydrolyzed at beverage pH, this sample also contains oleanolic acid, plus other non-sugars, after it has been dissolved in a simulated beverage (pH 2.5).

The observations on hydrolysis explains why Eis *et al.*, (1952) found it necessary to add back a relatively large quantity of isolated floc material before observing flocculation; probably sufficient was added back to form a haze rather than a true floc. The isolated floc material here appears to have been largely oleanolic acid, not saponins. Oleanolic acid itself is relatively insoluble in water, but becomes solubilized when sucrose is added.

The authors have, in past work (Roberts *et al.*, 1996; Clarke *et al.*, 1992), observed that isolated beet sugar floc (from beverage), identified as beet sugar-sourced by the high level of raffinose present, contained beet cell wall polysaccharide with galacturonic acid residues, and protein. The polysaccharide (given the trivial name Indigenous Beet Polysaccharide, IBP) (Roberts, *et al.* 1996; Clarke *et al.*, 1992) is comparable to the cell wall sugarcane polysaccharide, containing glucuronic acid groups, that can cause acid beverage floc when in combination with a protein. At beverage pH, the acid groups become negatively charged, the protein groups become positively charged; charge attraction brings the molecules together to form first a coacervate and then a flocculating network that entraps colloidal and suspended material to form a visible floc. This process is outlined in Figure 3.

The authors propose that a similar mechanism can be responsible for beet sugar floc: a carboxylic-acid containing molecule, saponin (a form that contains glucuronic acid), oleanolic acid, or cell wall polysaccharide, becomes negatively charged at low pH; an amino-group containing molecule (protein, peptide, or other) becomes positively charged. The two come together from charge attraction to initiate a floc network. This explanation accounts for the observance of floc without saponin present because another negatively charged molecule can be participating (oleanolic acid). This mechanism accounts for the presence of saponin without floc, if insufficient protein or positively charged amino group is present. It also accounts for the presence of floc without saponin, if oleanolic acid or IBP is forming a coacervate with protein.

Floc can be made in non-floccing sugars (beet or cane) by the addition of oleanolic acid and protein, as shown in Table 1. These observations provide information that oleanolic acid and protein/peptide are causative factors of acid beverage floc.

SAPONIN TESTS

All the postulates and observations in the literature involve validity of saponin tests. The traditional tests must be re-evaluated using instrumental analysis to distinguish between saponin and oleanolic acid. Literature discussion on the most frequently used test, the antimony pentachloride test, points out that its color reaction is not specific for triterpenes (van der Poel *et al.*, 1966). It is obviously then not specific for saponin versus oleanolic acid. Observations by the current authors indicate that the antimony pentachloride procedure is a test for oleanolic acid, not for saponins.

REMOVAL OF FLOC AND FLOC CAUSING COMPONENTS

Experience with cane sugar ABF has shown that components can be removed in process by tight pressure filtration (e.g. filter aid grade Hiflo Super Cel™ or finer), preferably at 60-65 °C. This low temperature is

a production problem, but ensures product quality. It is anticipated that similar filtration will remove the oleanolic acid, protein and other floc-causers from beet sugar.

Manufacturing lore has held that maintenance of processing pH above 8.5 after juice purification will prevent floc formation. It is herein proposed that at pH's above 8.5, the oleanolic acid is in the form of a calcium complex, or complex salt, and is removed during filtration and also possibly as evaporator scale. Therefore the floc causative agents are not transferred with the sugar, but removed from thick juice. When magnesium oxide is used in place of lime (Magox process), floc problems have been reported to become more frequent. Magnesium requires a pH much higher than 8.5 to form complexes, and so floc-forming oleanolic acid and other entities are not removed as complexes or salts in the Magox process, but remain in thick juice and pass on into sugar.

The growing use of membrane filtration processes will reduce the incidence of acid beverage floc formation because these membranes eliminate protein and other large organic molecules from sugar. The use of molasses desugarization or of softening processes, especially at high pH, should also reduce the incidence of ABF by removing the causative factors.

CONCLUSIONS

Comparison of traditional aqueous acid extracts of "saponin" from sugarbeet substrates with methanol extraction has shown that aqueous extraction yields only the aglycone of beet saponins, oleanolic acid. Chromatographic and mass spectrometric evidence support this. A re-examination of the relationship between saponin and oleanolic acid and "saponin" - caused problems, acid beverage floc and foaming, is required.

Acid beverage floc can be formed in simulated beverages by the addition of oleanolic acid and a protein/peptide.

It is proposed that floc formation has two causative factors: presence of a molecule that is negatively charged at beverage pH (saponin, oleanolic acid, or cell wall polysaccharide) and a molecule that is positively charged at low pH (protein or peptide); the two molecules come together in solution through charge attraction to form a coacervate that develops into a floc network. Development of quantitative tests for causative factors oleanolic acid and protein/peptide is in progress.

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Table 1. Determination of floc formation. Sample preparation: 240 g sugar, 500 ml water, 60 ml formaldehyde, pH = 2-3, 10 days. If added: oleanolic acid, 5 mg; protein, 5 mg. This preparation was found to be the test that most readily showed floc.

SUGAR	ADDITIONS	RESULTS
Beet, non-floccing		no floc
Beet, non-floccing	methanol extract	floc
Beet, non-floccing	oleanolic acid	floc
Beet, non-floccing	protein	no floc
Beet, non-floccing	oleanolic acid + protein	heavy floc
Beet, floc positive		fine floc
Beet, floc positive	saponin	fine floc
Beet, floc positive	oleanolic acid	heavy floc
Cane, non-floccing		no floc
Cane, non-floccing	oleanolic acid + amylase	fine floc
Cane, non-floccing	oleanolic acid + gelatin	coarse turbidity
Cane, non-floccing	oleanolic acid + beef serum	coarse turbidity
Cane, non-floccing	oleanolic acid + dextranase	fine floc
Cane, non-floccing	oleanolic acid + invertase	fine floc



Figure 1. (left) Non-floccing beet sugar in a floc test sample, (right) beet sugar showing acid beverage floc in a floc test sample.

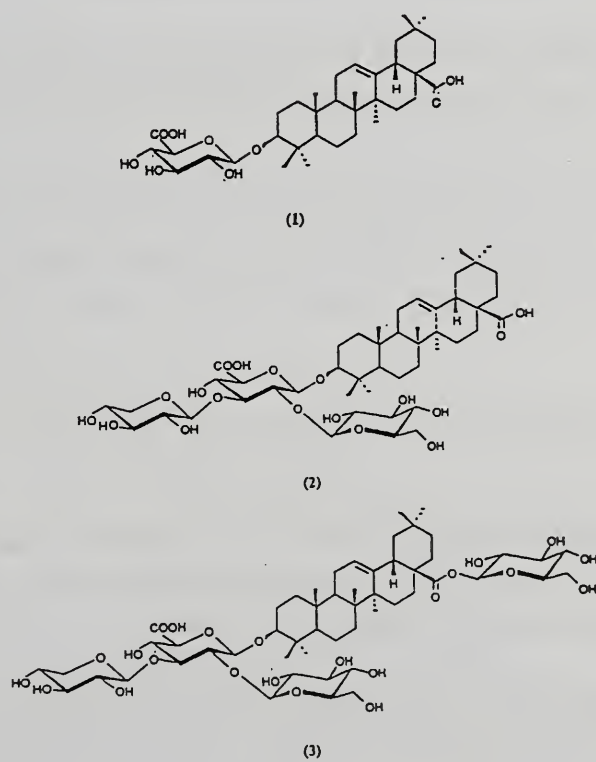


Figure 2. Three saponins of sugarbeet

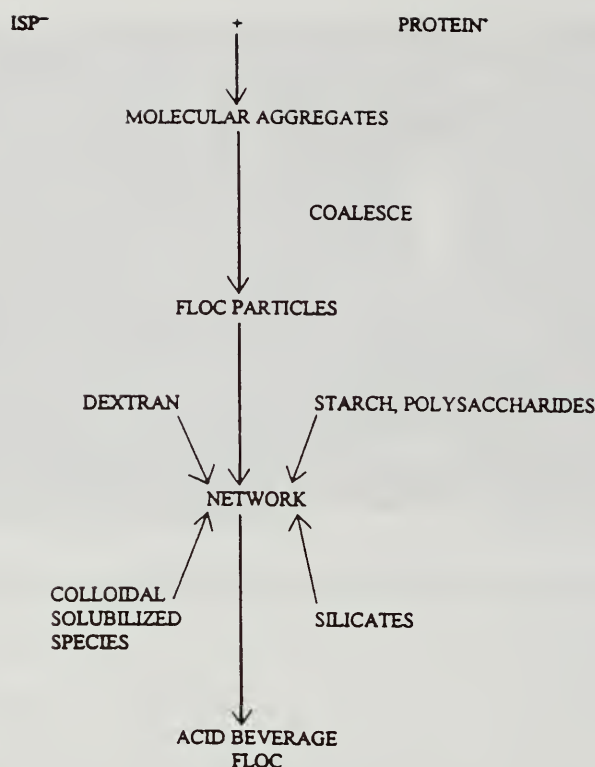


Figure 3. Schematic outline of formation of acid beverage floc in cane sugar systems. Any negatively-charged polysaccharide may react in this way with a protein (e.g. enzyme). (ISP = indigenous sugarcane polysaccharide)

DISCUSSION

Question: Is oleanolic acid a strong acid? Is it negatively charged at pH 2?

Clarke: In water I do not suppose it is. We did moving boundary electrophoresis where you have a cathode and anode and observed the oleanolic acid/sugar solution. It was not very soluble under these circumstances, and you could clearly see that it moved to the positively charged anode at pH 2. I do not think it would in water alone, but sugar, being a weak acid, changes these things.

Question: Have you ever treated a saponin floc which would have oleanolic acid and protein with a protease to see if it destroys the floc?

Clarke: We have not yet tried enzymes on beet floc although we have on cane floc. That is a good idea.

Question: Is there any evidence in floc from beet sugar that there is some kind of protein present?

Clarke: Yes, we have found that.

Question: In addition, you did some very interesting experiments adding different proteins to induce floc. Is it possible to isolate the high molecular weight material from sugar by dialysis, concentrate it, and then add it to a non-floccing sugar?

Clarke: Didn't we isolate some for you?

Question: Well, yes, but I have not had a chance to work on it.

Clarke: If you still have the material, perhaps you could try it and let us all know the result.

Question: I am asked occasionally from all around the world how to get rid of floc in both beet and cane, and the advice I have given is to do a light carbon polish. Would you comment on that and do you have any experience with how well that works? Secondly, you said that tight filtration will get it out. Would you elaborate on that?

Clarke: Regarding carbon, the experience at Spreckels Sugar (and I do not know anyone else who has tried activated granular carbon - this is thermally regenerable carbon which is more economic for a large plant) was that whatever was causing the floc was removed. I think that is because it was such thorough filtration. By a light carbon polish, I assume you mean putting up to 0.5% powdered carbon in a press filter?

Question: I would be looking at much lower than that. I think we would be looking at 0.05 to 0.1% by weight of sugar.

Clarke: Of course, that is going to depend on the quality of the sugar you put in it. If such a light carbon treatment is going to make a difference, it is not a very high quality sugar to begin with. I think treatment would have to be on an individual basis, determined as needed. If you are dealing with refined sugars from a cane refinery or high quality beet whites, that is probably not going to make much difference.

Question: How much carbon, in general, would do the job?

Clarke: Charlie Schmalz has an answer for that, I believe.

Comment: When U & I Sugar used to make a type 50 liquid sugar from a sugar with a floc propensity, we used to produce about 50,000 lbs of sugar in 7,000 gallon batches, and we put 50 lbs of activated carbon, typically Darco S-51, into the batch. It served a dual purpose. It produced an almost water-white liquid sugar, and it completely removed any floc problems at the same time.

Question: I am curious about the tight filtration. I know it works on cane, but what about beet?

Clarke: We have had some reports that filtration has to be below 70°C and at least a good medium grade filter aid, like HiFlow Super Cel or better. There has been some success. Again, I think it is just a measure of the total amount of filtration you put the sugar through. Certainly, neither the oleanolic acid nor the

S.P.R.I.

protein are going to be in particle form, but all of the floc initiators are soluble materials. None of them are insoluble. This is true for both cane and beet, and explains why they can get through the process.

Question: Do we have any idea where the oleanolic acid comes from? Is it under the peel of the sugarbeet?

Clarke: Yes - it is part of the saponins that are just under the peel of the beet, and are hydrolyzed in processing or in the acid solution in the floc test.

Question: Have you quantitated the amount of oleanolic acid that is needed to promote floc?

Clarke: We tried from 1-10 ppm of oleanolic acid on sugar, and 1 ppm did not cause floc, but 2 ppm did. I should mention that ultrafiltration might help a lot also, now that it is becoming more prevalent.

Question: The old bottler's standard was, I believe, 3 ppm "floc" and we found then that about 2 ppm saponin would give floc. We figured that only about 50% of what we saw as saponin was actually saponin.

Clarke: Since we know now that what was called saponin in those isolation procedures is actually oleanolic acid, that comes out to the same thing.

REPORT ON TREATMENT OF SUGARCANE JUICE WITH DISC-STACK CENTRIFUGATION

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INTRODUCTION

Work on membranes by the SPRI group has indicated that membrane fouling materials consist of: (1) soluble polysaccharides, high molecular weight colorants and other large molecules, (2) colloidal complexes, and (3) finely divided solid material suspended in solution. Earlier work (Devereux and Clarke, 1984) had shown that filter blocking particles were about $2\ \mu$ in diameter. Generally, these materials are not charged, and therefore are more difficult to remove by usual processes. The removal of suspended solids from the process stream offers the added potential benefit of removal of soluble high molecular weight material by physical absorption or chemical complexation.

In a search for a physical separation system to remove fine suspended solids and colloidal materials, Alfa Laval (Tumba, Sweden) was consulted. Alfa Laval equipment, of the disc-stack centrifuge type, is used in molasses desludging before molasses fermentation, but has not otherwise been employed in sugar manufacture. It does have applications experience in many other industries, including chemical, pharmaceutical, environmental, water treatment, and food.

With the increase in mechanical harvesting, there has been a concomitant increase in suspended solids in sugar, both direct white and raw sugar.

The goal of this study was to find a method to remove suspended solid material and fine particulate matter from sugarcane juice, to make it suitable for membrane filtration, for preparation of direct consumption sugar or to increase raw sugar yield at higher production efficiency.

The approaches for the study were several:

1. Consider separation before membranes; pretreatment, such as centrifugation.
2. Conduct feasibility discussions in Sweden and the United States with Alfa Laval, manufacturers of industrial centrifuge equipment.
3. Conduct bench-scale tests at Alfa Laval (U.S.) on fresh cane juice and syrup samples.
4. Conduct on-line testing of process equipment at a cooperating factory in Louisiana.

EXPERIMENTAL WORK

Bench-scale tests

Laboratory scale trials were conducted at the Alfa Laval Centrifuge Demonstration Laboratory in Warminster, PA, on fresh samples of sugarcane juices. It is emphasized here that only fresh juice was examined, because the freezing process changes the characteristics of the juice in, as yet not well understood ways, and causes clarification (or separation of solids) to proceed much more rapidly and efficiently than it does in the fresh state. Mixed, crusher and clarified juice and evaporator syrup, freshly obtained from a mill in Louisiana and maintained cool in an ice chest during transportation, was taken by S.P.R.I. personnel to the Warminster laboratory.

Trials were conducted with both a disc-stack centrifuge (Figure 1) and a high speed decanter separator (Figure 2). Separation of suspended solids from whole mixed juice and crusher juice could not be achieved with either system alone, but required both types of separator in series, with the decanter first, because of the wide range of particle sizes in mixed or crusher juices.

Cane juice after standard factory clarification (settling clarification with lime and polyacrylamide added to mixed juice) was, however, successfully separated by the disc-stack centrifuge alone, and so the decision was made to run pilot trials at a factory on clarified cane juice.

Evaporator syrup could also be successfully separated by disc-stack centrifuge alone, but because of the high solids content, required too long a treatment time.

Pilot tests on clarified juice

Pilot scale tests on clarified juice were carried out on December 16th and 17th, 1997, at the M.A. Patout and Son factory in Patoutville, Louisiana, to determine the extent of solids reduction from treatment of hot clarified juice. An Alfa Laval disc-stack centrifuge Model 403, 60-disc machine, was set up on line on clarified juice. The clarified juice results from simple settling defecation of mixed juice with lime, phosphoric acid and water soluble organic polymers. Although the flocculation process removes much turbidity from the mixed juice, a large amount of suspended colloidal solids remain, which could, heretofore, only be removed by microfiltration. The installation was a small model, running only a few gallons per minute. However, experience has shown that scale-up is effectively linear.

MATERIALS AND METHODS

The treated juices were tested to determine the degree of improvement for subsequent membrane filtration treatment. In the current work, a comparison of the colloidal filtration characteristics (0.2 μ average pore size or approximately 2 million Dalton nominal molecular weight cut-off ceramic filter) of supernatant effluent from a 60-disc continuous flow centrifuge was made for clarified juice at rates of 0.5 and 1.0 gallon per minute at a nominal temperature of 200° C. Both the feed clarified juice, the supernatant and the sludge

coming out of the centrifuge were taken for analysis of color and turbidity. The solids sludge portion can be sent to rotary vacuum mud filters. Some supernatant was retained for ultrafiltration testing.

The suspended solid is measurable by spectrophotometric turbidity measurements. Turbidity measurement was determined by subtracting the ICU color of the filtered solution from the ICU of the unfiltered solution, both measured at 420 nm.

Because of the widespread use of 720 nm to measure turbidity in parts of the industry, measurements were made at that wavelength also. Since there is only a low baseline of color absorption at 720 nm in the visible range, a direct reading can be made for turbidity as above at 720 nm and the turbidity absorbance units calculated for the suspended particulates alone since color is not the dominant absorbing species at 720 nm. The reading of turbidity at 720 nm is used as an alternative to the 420 nm reading. The latter is preferred because the 720 nm reading can sometimes be negative when the turbidity is very low.

RESULTS

Table 1 shows the removal of turbidity by disc-stack centrifuge, measured either at 420 nm or 720 nm. Samples were compared to the turbidity of the feed coming from the clarifier at the time of taking the designated flow rate samples. It is noted that the 420 nm and 720 nm results were roughly comparable, with the 720 nm measurements showing slightly higher turbidity removal.

Results indicated that from 67% to 80% of the fine suspended solids were removed from the clarified juice by treatment at a flow rate of 1 gal/min. A lower flow rate of 0.5 gal/min did not result in significant improvement. However, as shown below, the lower flow rate did result in higher flow rates through ultrafiltration membranes.

Ultrafiltration membrane test

To determine the relative performance on ultrafiltration of treated juice, both treated and untreated clarified juice was filtered on the ceramic membrane pilot equipment described above. The results are summarized in Table 2. It is evident that the ultrafiltration flow rates were increased from 66% (1 gal/min) to 2.3 times (0.5 gal/min) compared to the untreated juice, indicating that the membrane fouling material was greatly reduced after disc-stack centrifuge treatment.

BENEFITS OF THE PROCESS

Evaporation: The 70% to 80% reduction in suspended solids (turbidity) will decrease juice viscosity and evaporator scale formation and should improve efficiency by 10% to 20%.

Crystallization: Reduction in suspended solids and the corresponding decrease in viscosity will improve crystallization rate by an estimated minimum of 10% of time. The treatment will improve crystal yield by an estimated 10% to 20%, and will improve sugar quality by reducing turbidity (sediment) by 80% to 90%.

Membrane filtration: Tests of membrane filtration of clarified juice, through a ceramic type filter of $0.2\ \mu$ average pore size showed an improvement in flux of 80% to 100% above that of untreated clarified juice. Cycle times for membranes (between cleanings) will, therefore, approximately double.

CONCLUSIONS

Reduction of the turbidity in clarified juice by high speed centrifugation results in a clarified juice with much less burden of solids to be carried forward to evaporators and pans. The improvement in the quality of the juice results in lowered maintenance costs and higher yields of higher quality crystallized sugar.

A much earlier study (Fort and Smith, 1954) demonstrated the advantages of improved clarification by centrifuging clarified juice, but concluded that centrifuging of clarified juice was not practical except as a research tool. Times and economies have changed and it is possible that this type of treatment will now become a useful and economical tool for improved production of sugar. More and larger scale tests at cane factories and beet factories are envisioned.

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Table 1. Removal of turbidity by disc-stack centrifuge (12-17-97).

Sample and throughput	420 nm		720 nm	
	% removed by centrifuging	% remaining after centrifuging	% removed by centrifuging	% remaining after centrifuging
Supernatant at 0.5 gal/min	70	30	79.3	20.7
Supernatant at 1 gal/min	67.5	32.5	82.1	17.9
Supernatant at 2 gal/min	61.3	38.7	52.85	47.15

Table 2. Test of ultrafiltration membrane with disc-stack treated clarified juice.

Sample	Normalized flow rates
Untreated clarified juice	1.00
Juice treated at 0.5 gal/min	2.32
Juice treated at 1 gal/min	1.66

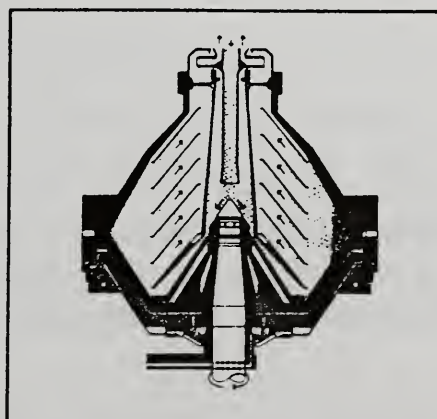


Figure 1. Disc-stack centrifuge.



Figure 2. Decanter centrifuge.

DISCUSSION

Question: How good was the clarified juice that you used in your test compared to the average clarified factory juice? My experience is that the turbidity can vary enormously between clarified juices, from one day to the next due to weather conditions and so on. You may just be taking a poorly clarified juice and turning it into a good clarified juice.

Clarke: The conditions were Louisiana conditions, which are not the best in the world, but they were the best of Louisiana conditions. We had very good weather, no frozen cane, no particularly bad samples. We had hoped, in fact, to get some poorer conditions so we would have those to contrast with, but it was not to be. You have made a good point, though.

Vercellotti: If I may add to that, we did run for three days consecutively, and there was a whole variety of cane material going into the factory - whole stalk, billets, trash - it was quite a mix. I think if we were going to see really bad stuff going through the clarifier, we would have seen it in those three days.

Question: Were you running turbulent flow or laminar flow in your cross flow experiment, and did you calculate a Reynolds number?

Vercellotti: We were running turbulent flow, but we did not calculate a Reynolds number.

Question: The reason I asked is that the ability to handle those balances would be very subject to what kind of linear velocity you are running across the membrane.

Vercellotti: On the other hand, it is not that kind of data that we are trying to generate. We are trying to find out what plugs up those membranes. And as I mentioned in my earlier paper on beet juice, there is stuff in the juice that really plugs up the membrane.

Question: That may be so under the conditions you are studying, but the intent should be to find a practical way to operate these membrane systems. Running at a higher linear velocity might have a better effect.

Vercellotti: At this time, our intent is to do the organic chemistry on the composition of the membrane-fouling materials in the juices and molasses.

Clarke: We were trying to find a way to remove some of the material we know blocks the membranes. We would be happy to try out other company membranes, if they are sponsors of S.P.R.I.

Question: As I mentioned in my talk this morning, we are working to improve the mechanization in Colombia. We are very interested in your doing this kind of work.

In Cali we have a 5,000 tons per day mill. If we were to install this type of centrifuge system, would it be better operated on mixed juice, crushed juice, or something else, like clarified juice or even syrup?

Clarke: The bench scale tests showed that clarified juice could be treated successfully using the disk stack centrifuge only, and that is what we tried at the factory. We have not yet tried mixed juice on a factory scale, but we believe that would take, as the company recommended, a two-stage separation of a decanter centrifuge first followed by the disk stack centrifuge. That means more equipment. Your factory, at 5,000 T/day is roughly half the size of the factory at which we ran these tests. We did not try syrup because it took so much longer to clean up that we did not think it would be economical and would require too much more equipment.

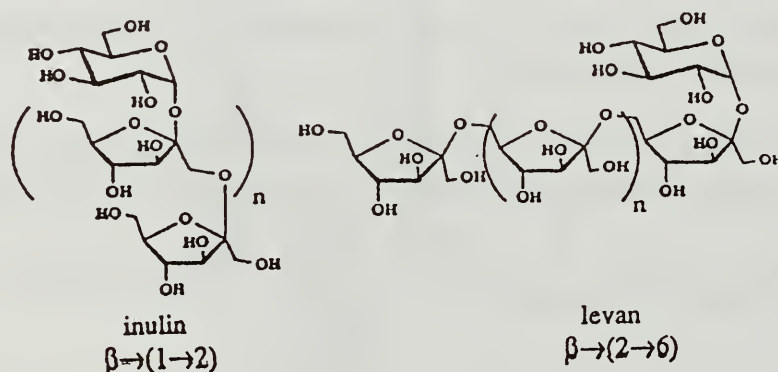
POSTER

MICROBIAL POLYSACCHARIDES FROM SUCROSE

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INTRODUCTION

Levans are polymers of fructose, with $\beta\rightarrow(2\rightarrow6)$ linked backbone structure. Levans are generally high molecular weight polysaccharide products of microorganisms. In contrast, inulin, another class of polymers of fructose, are characterized by a $\beta\rightarrow(1\rightarrow2)$ linked backbone structure, and are generally found in plants (chicory, Jerusalem artichoke, onion, among others). Some low molecular weight levans are found in grasses; these compounds are often called fructans.



Levans are made by many bacterial species and other microorganisms, including: *Acetobacter* spp.; *Achromobacter* spp.; *Actinomycenes* spp.; *Aerobacter levanicum* and other species; *Aspergillus* spp.; *Bacillus polymyxa*; *B. licheniformis*; *B. macerans*; *B. mesentericus*; *B. vulgatus*; *B. megatherium*; *B. subtilis* and other *Bacillus* spp.; *Corynebacterium laevaniformans*; *Erwinia herbicola*; *Leuconostoc mesenteroides*; *Phytobacterium vitrosus*; *Pseudomonas Fluorescens* and other Pseudomonads; *Phytomonas pruni*; *Streptococcus mutans*, *S. bovis* and *S. salivarius*; *Xanthomontas campestris* and *X. pruni*; and *Zymomonas mobilis*.

In an ongoing program to develop new products from the renewable agricultural resources of sugar producing crops, a search for microorganisms to produce compounds for industrial use led to the isolation of a bacterium that produces high molecular weight levan in high yield (1, 2). The microorganism is a species of *Bacillus polymyxa*. Its culture, the production of the levan, and structural identification are now in the literature (1, 2, 3). *B. polymyxa* produces levan, commonly known as polyfructose, from sucrose only, not from glucose or other simple sugars. Yields are up to 40% on sucrose from sucrose solution or sucrose in cane and beet juices, syrups and molasses.

Hydrolysis of levan: fructose and fractions

Acid hydrolysis

Levan is readily hydrolyzed by acids and enzymes to either fructose or low molecular weight levans. Fructose is a well known sweetener. Levan is faintly sweet.

At pH 3.5 or below, levan is hydrolyzed to fructose, yielding a sweet solution. The addition of heat greatly increases the rate of hydrolysis.

Acid hydrolysis to fructose of a 10% aqueous solution of levan.

Conditions	Time	Temperature	% fructose by HPLC
0.5% citric acid	48 hours	r.t.	
	2 minutes	microwave	33
	5 minutes	microwave	76
	7 minutes	microwave	100
0.5% ascorbic acid	15 minutes	100°C	100

Because this levan is not hygroscopic, it provides a means to store fructose in a dry state; crystalline fructose picks up moisture quickly. Addition of flavor, and subsequent acid hydrolysis, will produce a sweet, flavored solution.

Enzyme hydrolysis

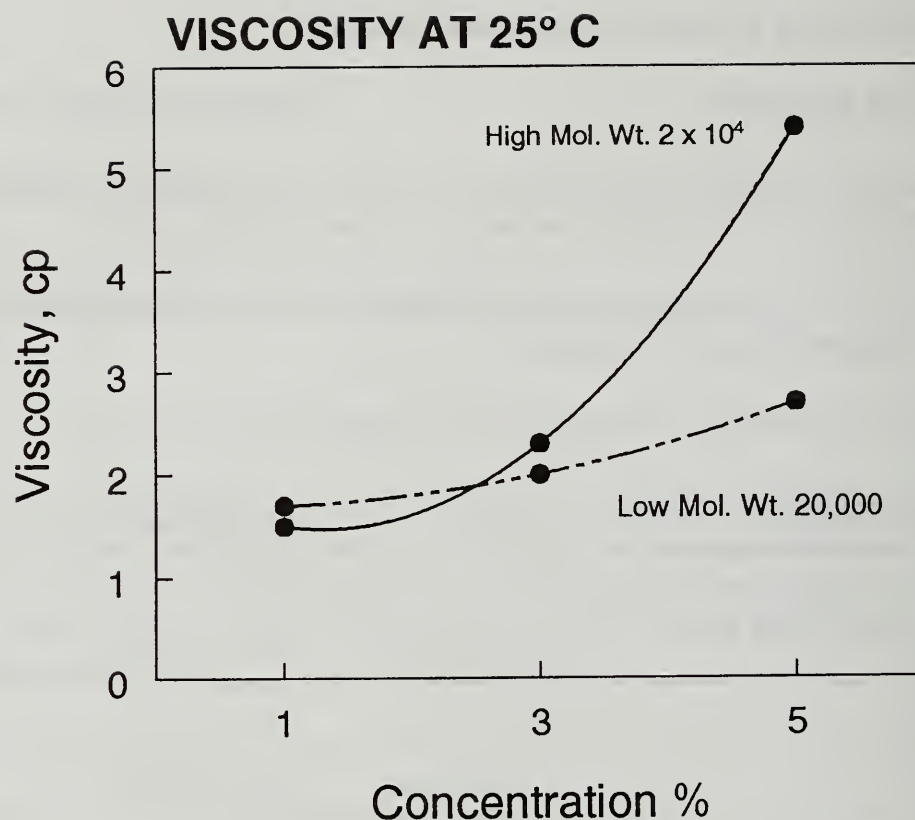
Several commercially available enzymes, including Gamanase (Novo Biochemicals, Inc.) hydrolyzed the levan to a smaller levan, with molecular weight average 20,000 Da. The original levan, or polyfructose, has a molecular weight of 2×10^6 Da, with a very varied spread of molecular weight; it has some 12% β -(2 \rightarrow 1) branching and 13% terminal groups (1). The lower molecular weight levan has been shown (1) to maintain a similar structure. The lower molecular weight compound is, however, much more soluble than the high, giving a clear solution.

The low molecular weight levan, also hygroscopic, hydrolyzes more rapidly than the high molecular weight polyfructose. Viscosity of the smaller molecule is less, and optical rotation is lower.

Hydrolysis of levans at 65°C
in 0.5% oxalic acid

Time (min)	% fructose	
	Low Mol. Wt. (20,000)	High Mol. Wt. 2×10^6
0	0	0
10	29	24
20	68	46
30	96	68
40	100	88
60		92
80		100

	wD^{24}	m.p.(°C)
High Mol. Wt. (2×10^6 daltons)	-42.3°	>200
Low Mol. Wt. (20,000 daltons)	-62.2	92



Derivatives of levan

Several derivatives of polyfructose have been prepared. Of interest is the phosphate derivative, prepared by phosphorylation with PCl_3 or POCl_3 in pyridine, and separated as the sodium salt. Degree of phosphorylation of sodium levan phosphate products varies from 2% to 12 %. Nmr data indicate that phosphorylation occurs on C-1, until C-1 is fully phosphorylated, and then partial substitution occurs on C-3 and C-4.

Levan phosphate has the unusual property of causing water to gel and causing some organic solvents (including ethanol, other alcohols, DMSO), to gel, when added at levels of 15 to 10%.

A wide range of medical and food processing uses are envisaged for levan phosphate.

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2. Clarke, M. A., E. J. Roberts, W. S. Tsang, M. A. Godshall, Y. W. Han, L. Kenne and B. Lindberg. (1990). Structural studies on a fructan from sugar beet and sugar cane juice. Proc. Conf. Sugar Proc. Res., pp. 139-146.
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POSTER

VERIFICATION OF SCREENING SIEVES

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ABSTRACT

This paper describes the methods and procedures used to establish tolerances and evaluate used screen sieves. Granulated sugar samples are passed over certified screens to establish "true" weight fractions. Sugar is retained and passed over new screens to establish minimum tolerances. Tests are repeated to establish statistical confidence.

By comparing the difference in results and calculating standard deviations, acceptable tolerances are developed for each screen size. Routine verification checks are performed as part of the ongoing maintenance of our Quality System. "Calibration" procedures and results are presented for review.

INTRODUCTION

Crystal size distribution is an important quality parameter in granulated sugar products, and of specific interest to many industrial users. Size distribution is important to industrial users for a variety of reasons:

- It affects the color distribution and appearance of dry blends.
- Through proper selection, it ensures that blended product is not prone to segregation in storage.
- It impacts dough spreads and ultimately the final product size in baking applications.
- It quantifies the level of fines present in the sugar which causes dust problems in handling and storage equipment.

In 1994, Lantic Sugar modified its existing Quality System with the objective of becoming certified to the ISO 9002: 1994 Quality Standard. With grists being an important specification of granulated sugar and calibration an important element of the ISO 9002 Standard, it was necessary to develop a method of evaluating screen sieves in use.

Initially several ISO certified facilities were contacted to determine if any "in-house" methods were available; however, none of them had developed a quantitative method of evaluating used screen sieves. Most facilities simply inspected their sieves on a routine basis and arbitrarily discarded them after a fixed period regardless of wear.

This paper introduces a simple and economical method of evaluating used sieves and describes how minimum standard tolerances are developed. Methods are based on fundamental statistical techniques and meet the requirements of the ISO 9002 Quality System.

Test methods, selected calibration data and observations are presented for review and discussion.

METHODS

1- Sieving method

All sieve tests were performed according to ICUMSA, Method GS2-37 (1994): The Determination of Particle Size Distribution of White Sugar by Sieving. Mean aperture (MA) and coefficient of variation (CV) values were calculated using the RENS method (ICUMSA, GS2-37 (1994), section #3 of appendix). New sieves were washed with a soft soap, air dried and placed on a grounded metallic surface to reduce the static charge associated with plastic wrapping. All sieves sizes in this report refer to the U.S. Standard Series.

2- Recovery method for multiple sieve tests on a given sample

Initial sieve tests were performed according to item 1 above. Upon completion of the test, sugars retained on the sieve(s) and bottom pan were emptied onto a piece of smooth paper. Sieves were brushed gently to remove any sugar crystals trapped in the openings. The entire sample was then weighed and re-tested on the next sieve(s) to be used.

3- Splitting of sugar samples

Sugar samples were split using a standard riffle device.

4- Type of sugar used for screen tests

Each type of sugar used for screen comparisons was selected on the basis of average crystal size compared to screen opening. Sugar types were chosen to achieve a minimal percentage retained without flooding the cloth opening (25 to 50 percent retained was considered optimal). For some sugars a scalping screen was necessary to achieve the optimal quantity of retained sugar. The type of sugar used to establish tolerances was the same as that used to evaluate used sieves. A complete list of sugar types for each size is included in Appendix A.

DISCUSSION

One of the key requirements of the ISO 9002 Quality System is that the calibration of critical measuring equipment is traceable to national standards. In Lantic's specific case, traceable certified screens (NIST Certified) were obtained and used as a basis for comparison. Our registrar confirmed that the traceability

and certification of the screens met ISO 9002 requirements. Upon confirmation, a method of comparing used in-house screens to the traceable screens for periodic validation checks was developed.

Initially some refined granulated samples were separated on a standard riffing (separation) device and screened consecutively on a set of traceable screens. Table 1 shows two typical sugar samples that have been riffled into five separate samples and screened. As shown, there is significant variation between test results. The mean aperture of the Coarse sample ranged between 1433 and 1474 microns and the mean aperture of the Fine sugar sample ranged between 450 and 461 microns.

In order to determine the impact of riffing on variation, sugar samples were passed over a set of traceable screens, recovered, and re-screened five consecutive times. The "recovery" method produced much more consistent results as shown in Table 2. It can be seen from the data that weight distribution is virtually identical for all five tests. The mean aperture for the Coarse sample ranged between 1319 and 1320 microns and the mean aperture for the Fine sugar sample ranged between 447 and 450 microns. The range and consistency of MA is significantly better than results obtained by riffing.

Results also indicate that there is no significant change or destruction of sugar that has been gristed and recovered. For both sugar samples there was no measurable reduction in MA or increase in fines between the first and last test. In both cases, the MA actually increased marginally. Tests were repeated for different types of granulated sugar with results consistent with the above.

Table 3 shows a comparison of variability for sugar samples that are recovered instead of riffled. For each test run, a single sample has been riffled and screened over a traceable 50 U.S. sieve five times. The third riffled sample has been recovered and re-screened an additional four times. The recovery method produced standard deviations of 0.13 and 0.045 versus standard deviations of 2.96 and 3.35 for the riffle method. It is noteworthy that results are consistent with one another and were performed in two independent labs with independent sugar samples. It was thus concluded that riffing was the main source of variation and that the gristing process and sample integrity were remarkably consistent.

In summary, three important observations had been made:

1. The separation of sugar using a standard riffing device contributes significantly to variation in size distribution.
2. With a given sugar sample, the standard gristing procedure produces consistent results.
3. There is no significant size change or destruction of granulated sugar that has been screened and recovered up to five consecutive times. This is true for standard Fine, Extra-Fine, and large grained specialty products.

Recognizing that the recovery / re-gristing method produced consistent results we were able to develop a method of comparing used in-house screens to the traceable certified screens.

In order to determine the standard variation between the traceable screens and in-house screens, a complete set of new in-house screens were ordered. Each new screen, ASTM certified, was compared to the traceable screen of the same mesh size to determine the magnitude of variation.

Comparisons were completed by screening granulated sugar samples across the traceable screen, recovering the sugar and re-screening across the new screen. Tests were repeated a minimum of 20 times with 20 different sugar samples for each screen size to determine the average percent difference and standard deviation. The traceable screen result was chosen as the “true” result in the “% Difference” calculation.

Table 4 shows the variation between the traceable screens and the new screens for the 30 and 40 U.S. screen sizes. The average differences were 1.3% and 1.4% with standard deviations of 0.42% and 0.92% respectively.

In order to determine whether the comparison data was normal, the percent differences were plotted on normal probability paper as shown in Figures 1 and 2. The high degree of linearity for both of these plots confirmed that the comparison data was indeed distributed normally. Similar results were achieved for all screen sizes; however, omitted for reasons of brevity.

Recognizing that the distribution was normal, the standard deviation between newly purchased ASTM screens and traceable certified NIST screens was quantified.

The minimum acceptable tolerance for each screen size was calculated using the following equation:

$$\text{Minimum Tolerance} = \text{Avg \% Difference} + 3 \times \text{Standard Deviation}$$

Statistically, this “Minimum Tolerance” represents the expected variation between a newly purchased screen and a traceable screen 99.75% of the time. Any variation exceeding the minimum tolerance is an indication of screen wear.

In determining the acceptable tolerance for each screen size a % Difference equal to or greater than the calculated minimum tolerance was chosen by the Quality Assurance Department. For all screen sizes, the minimum acceptable tolerance was no lower than the Average % Difference + 5%. A complete list of minimum and acceptable tolerances for each screen size is shown in Table 5.

Assuming that a given set of screens were at the limit of their tolerances, the impact on product MA can be predicted. The maximum variation would occur when $+d_{50}$ screens were at the maximum tolerance and $-d_{50}$ screens were at minimum tolerance. Table 6 shows the predicted range of MA for three typical refined products (Fine, Extra-Fine and Powder). As shown, the MA for all samples varies by 3.6% or less even if screens are at the outside limits of their tolerance. Figures 3, 4 and 5 show the impact of the acceptable tolerances on the entire size distribution for the same three samples. It is evident from these graphs that even at maximum tolerance, the impact on overall size distribution is negligible.

Table 7 shows typical screen verification data for two of our used screen sieves since the implementation of the verification procedures. Both screens have remained within acceptable tolerance for the 27 month period and do not appear to be at risk of rejection in the near future. Similar results have been achieved for all screen sizes with no rejection occurring outside of some initial rejections of visibly worn sieves.

In reviewing the data for screen no. 40-10, we see that a significant change occurred between February 1997 and May 1997. It is believed that the change is a result of washing the screen rather than screen wear. In either case, the screen remains well within acceptable tolerance.

The screen verification instruction and a sample "calibration" record are included in Appendices A and B for review.

Screens verification procedures were initially developed to meet the requirements of the ISO 9002 Quality Standard. These have, however, resulted in side benefits. Since the implementation of these procedures, there is an increased level of confidence in test results and in the integrity of our used sieves. Furthermore, there is a cost benefit of performing routine verifications as opposed to using traceable screens throughout the facility. Table 8 shows an economic comparison of the validation method versus the arbitrary replacement / recertification of a complete set of traceable screens every three years. A cost saving of more than C\$ 5000 is estimated.

Throughout the refinery, approximately 50 sieves are used by Production and Quality Assurance for process control and for product approval. It is estimated that over the past 27 months, some of these screens have been used as many as 3000 times without any sign of approaching their acceptable tolerance. In light of this, it is believed that the estimated savings are conservative and could be much higher.

CONCLUSIONS

The standard sieving process is highly repeatable provided that identical samples are used.

There is no measurable destruction or change in size distribution of sugar that has been screened and recovered five consecutive times. This holds true for all standard refined granulated products.

The separation of a sugar sample using a standard riffing device is not ideal and is the main cause of variation in size distribution analyses. Any sample that requires riffing will therefore be subject to this variation.

The periodic comparison of used sieves against traceable standards is a simple and effective method of monitoring wear and sieve integrity. The method meets the traceability requirements of ISO 9002: 1994 Quality Standard and is more economical than other known procedures.

Granulated sugar is not highly abrasive on sieves. Some sieves have been used as many as 3000 times with no indication of wear.

The minimum sieve tolerances calculated in this report are site specific. Any refinery wishing to use this method should develop tolerances with their own sieves and specific sugar samples.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contributions of Mr. Larry Brown for the countless number of sieve tests and technical support.

Table 1. Granulated sugar riffled consecutively and screened on a set of traceable sieves.

Coarse sugar sample	Percent weight retained				
	Test #				
Screen size	1	2	3	4	5
12 U.S.	22.2	26.5	24.9	24.7	26.4
16 U.S.	53.7	51.7	52.1	53.5	52.4
20 U.S.	20.8	18.4	20.0	19.3	18.1
30 U.S.	2.7	2.4	2.4	2.2	2.3
Through 30 U.S.	0.6	1.0	0.6	0.3	0.8
Total	100	100	100	100	100
MA ()	1433	1471	1457	1463	1474
CV (%)	24	25	24	23	24
Fine sugar sample	Percent weight retained				
	Test #				
Screen size	1	2	3	4	5
20 U.S.	0.1	0.1	0.1	0.1	0.1
30 U.S.	5.4	4.8	5.1	5.1	5.8
40 U.S.	56.0	54.9	56.2	56.4	57.7
50 U.S.	32.9	33.7	32.5	32.5	31.0
60 U.S.	3.7	4.3	4.0	4.0	3.6
80 U.S.	1.7	1.9	1.8	1.8	1.5
Through 80 U.S.	0.2	0.3	0.3	0.3	0.3
Total	100	100	100	100	100
MA ()	454	450	454	455	461
CV (%)	22	23	23	23	23

Table 2. Granulated sugar recovered consecutively and screened on a set of traceable sieves.

Coarse sugar sample	Percent weight retained				
	Test #				
Screen size	1	2	3	4	5
12 U.S.	12.7	12.6	12.6	12.7	12.7
16 U.S.	52.2	52.6	52.4	52.4	52.6
20 U.S.	28.7	28.3	28.5	28.3	28.2
30 U.S.	4.9	4.8	4.9	4.9	4.9
Through 30 U.S.	1.5	1.7	1.6	1.7	1.6
Total	100	100	100	100	100
MA ()	1320	1320	1319	1319	1320
CV (%)	25	25	25	25	25
Fine sugar sample	Percent weight retained				
	Test #				
Screen size	1	2	3	4	5
20 U.S.	0	0	0	0	0
30 U.S.	8.8	8.9	8.9	8.8	8.7
40 U.S.	50.2	51.0	50.7	50.9	51.1
50 U.S.	34.5	33.7	34.0	33.8	33.7
60 U.S.	4.2	4.1	4.1	4.1	4.2
80 U.S.	1.9	1.9	1.9	2	1.9
Through 80 U.S.	0.4	0.4	0.4	0.4	0.4
Total	100	100	100	100	100
MA ()	447	450	449	449	450
CV (%)	23	24	24	24	24

Table 3. Comparison of recovery verses riffling for an Extra-Fine sample screened on a 50 U.S. sieve.

Refinery A			
		% weight retained	
		Riffing	Recovery
Rifle #1		53.2	
Rifle #2		56.7	
Rifle #3		51.4	51.4
	Recovery #1		51.3
	Recovery #2		51.5
	Recovery #3		51.2
	Recovery #4		51.2
Rifle #4		50.9	
Rifle #5		48.8	
Average		52.2	51.3
Standard deviation		2.96	0.13
Refinery B			
		% weight retained	
		Riffing	Recovery
Rifle #1		49.6	
Rifle #2		56.8	
Rifle #3		52.8	52.8
	Recovery #1		52.8
	Recovery #2		52.8
	Recovery #3		52.8
	Recovery #4		52.7
Rifle #4		49.3	
Rifle #5		48.9	
Average		51.5	52.8
Standard deviation		3.35	0.045

Table 4. Variation data for establishing minimum tolerances on 30 and 40 U.S. sieves.

Run #	% difference - 30 U.S.	% difference - 40 U.S.
1	2.0%	0.7%
2	2.2%	1.9%
3	1.7%	2.1%
4	1.3%	2.7%
5	1.1%	1.4%
6	1.1%	1.8%
7	0.7%	1.6%
8	1.0%	2.6%
9	1.1%	2.8%
10	0.8%	1.7%
11	1.3%	3.1%
12	1.3%	0.0%
13	1.8%	1.4%
14	1.7%	1.1%
15	1.5%	1.0%
16	1.6%	0.3%
17	1.5%	1.6%
18	1.1%	1.0%
19	0.7%	0.3%
20	1.2%	1.6%
21	1.1%	0.7%
22	0.8%	-0.3%
23	0.9%	0.6%
Average % difference	1.3%	1.4%
Standard deviation	-.42%	0.92%
Average 3 X STD	1.3% 1.3	1.4% 2.8

Table 5. Calculated minimum and acceptable tolerances for periodic screen validations.

Screen size	Percent average of differences	Standard deviation of percent average differences	Calculated minimum tolerance (%)	Acceptable tolerance (%)
12 U.S.	3.7	1.64	3.7 ± 4.9	4.0 ± 5.0
16 U.S.	0.8	0.40	0.8 ± 1.2	1.0 ± 5.0
20 U.S.	1.6	0.90	0.6 ± 2.7	2.0 ± 5.0
30 U.S.	1.3	0.42	$1.3 \pm 1.3^*$	1.0 ± 5.0
40 U.S.	1.4	0.92	$1.4 \pm 2.8^*$	1.0 ± 5.0
50 U.S.	6.5	0.70	6.5 ± 2.1	7.0 ± 5.0
60 U.S.	5.0	1.4	5.0 ± 4.2	5.0 ± 5.0
70 U.S.	2.8	1.5	2.8 ± 4.5	3.0 ± 5.0
80 U.S.	0.6	0.5	0.6 ± 1.5	1.0 ± 5.0
100 U.S.	4.9	2.0	4.9 ± 6.0	5.0 ± 6.0
200 U.S.	1.2	0.9	1.2 ± 2.7	1.0 ± 5.0

* As per Table 4.

Table 6. The impact on MA if screens are at their maximum tolerance.

Types of sugars	U.S. screen	Tolerances	Typical Analysis				
			%	Adjusted to upper tolerance		Adjusted to lower tolerance	
F I N E	20	-3 to +7	0.10	+7	0.10	-3	0.10
	30	-4 to +6	4.80	+6	4.98	-4	4.62
	40	-4 to +6	49.65	+6	51.57	-4	45.33
	50	+2 to +12	28.67	+2	27.53	+12	32.22
	80	-4 to +6	15.88	-4	14.94	+6	16.89
	100	-1 to +11	0.50	-1	0.48	+11	0.45
	Through		0.40		0.39		0.39
	MA (μ)		424		430		413
	CV (%)		25		25		24
	Change (%)				1.2%		-2.4%
E F X I T N R E A	40	4 to +6	2.60	+6	2.62	-4	1.99
	50	+2 to +12	50.10	+12	53.26	+2	48.97
	60	0 to +10	25.00	0	23.73	+10	26.35
	80	-4 to +6	20.50	-4	18.68	+6	20.82
	100	-1 to +11	1.30	-1	1.25	+11	1.38
	Through		0.50		0.47		0.48
	MA (μ)		301		307		299
	CV (%)		20		21		20
	Change (%)				2.0%		-0.7%
P O W D E R	60	0 to +10	1.10	+10	1.18	0	1.06
	70	-2 to +8	15.03	+8	15.74	-2	14.11
	80	-4 to +6	34.97	+6	35.95	-4	34.84
	100	-1 to +11	29.96	-1	28.77	+11	31.86
	Through		18.94		18.37		18.14
	MA (μ)		180		181		179
	CV (%)		18		18		18
	Change (%)				0.006		-0.6%

Table 7. Refinery verification data for two typical sieves over a 27 month period.

Sieve number: 30-1 (30 U.S. sieve)			Screen number: 40-10 (40 U.S. sieve)		
Dates	Actual results	Acceptable tolerance	Dates	Actual results	Acceptable tolerance
Nov. 95	0.03	-4.0 to +6.0%	Nov. 95	0.028	-4.0 to +6.0%
Feb. 96	-0.2%	-4.0 to +6.0%	Feb. 96	0.04	-4.0 to +6.0%
May 96	-3.8%	-4.0 to +6.0%	May 96	0.04	-4.0 to +6.0%
Aug. 96	-3.3%	-4.0 to +6.0%	Aug. 96	+2.4	-4.0 to +6.0%
Nov. 96	-2.8%	-4.0 to +6.0%	Nov. 96	+4.3	-4.0 to +6.0%
Feb. 97	-3.8%	-4.0 to +6.0%	Feb. 97	+3.7	-4.0 to +6.0%
May 97	-0.1%	-4.0 to +6.0%	May 97	-0.9	-4.0 to +6.0%
Aug. 97	-1.2%	-4.0 to +6.0%	Aug. 97	+0.4	-4.0 to +6.0%
Nov. 97	-1.3%	-4.0 to +6.0%	Nov. 97	-1.3	-4.0 to +6.0%
Feb. 98	-2.3%	-4.0 to +6.0%	Feb. 98	+0.7	-4.0 to +6.0%

Table 8. Economic comparison of routine verification versus periodic replacement/recertification over a three year period.

		Cost per item (\$C)	Total (\$C)	Sub-Total (\$C)
1. Material				
Number of screens in usage	50			
Percentage initially rejected	X 10%			
Number of new screens required	= 5	\$65.00	\$325.00	
Number of new screens acquired for the purpose of establishing tolerances	11	\$65.00	\$715.00	
Number of certified screens bought	11	\$145.00	\$1,595.00	
Cost of recertification	11	\$80.00	\$880.00	
2. Manpower				
Estimation of 40 hours for periodical verification (excluding initial tests for the establishment of tolerances)		\$800.00		
For a three year period			\$2,400.00	
Sub-Total				\$5,915.00
3. Alternative Method				
Cost of buying certified screens	50	\$145.00	\$7,250.00	
Recertification after a 3 year period	50	\$80.00	\$4,000.00	
Sub-total				\$11,250.00
4. Saving				\$5,335.00

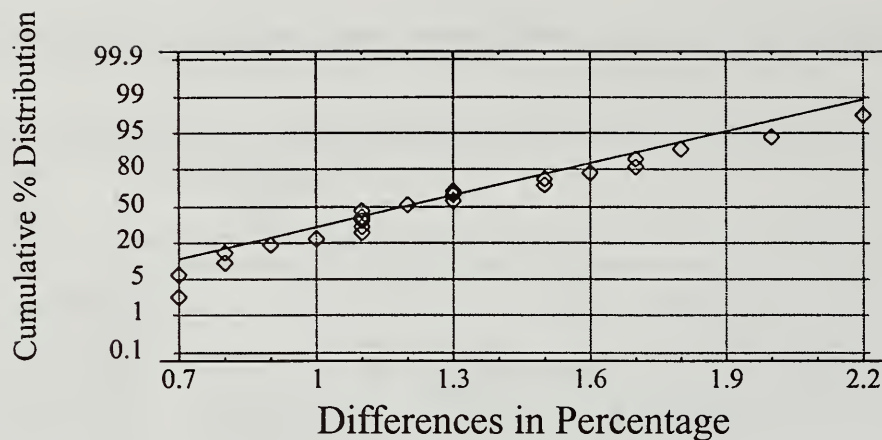


Figure 1. Normal probability distribution for the 30 U.S. screen variation data.

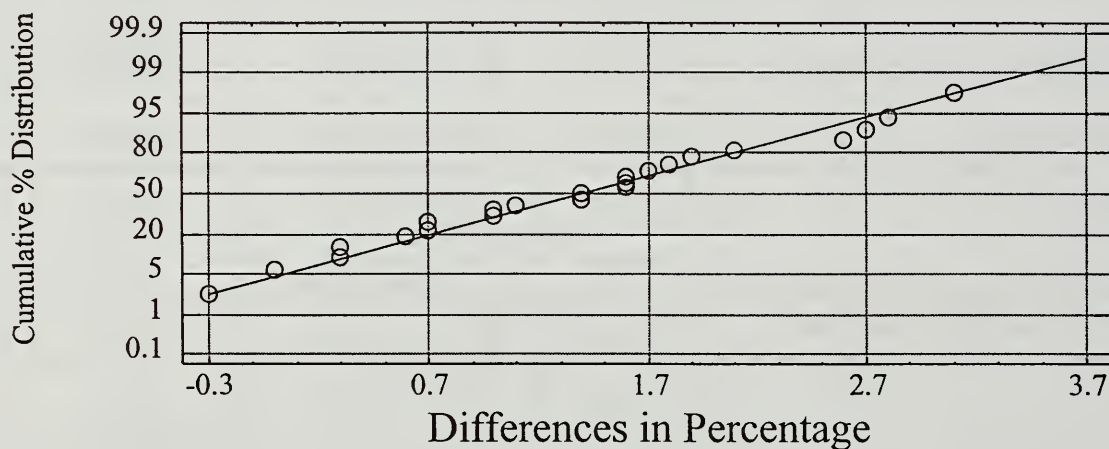


Figure 2. Normal probability distribution for the 40 U.S. screen variation data.

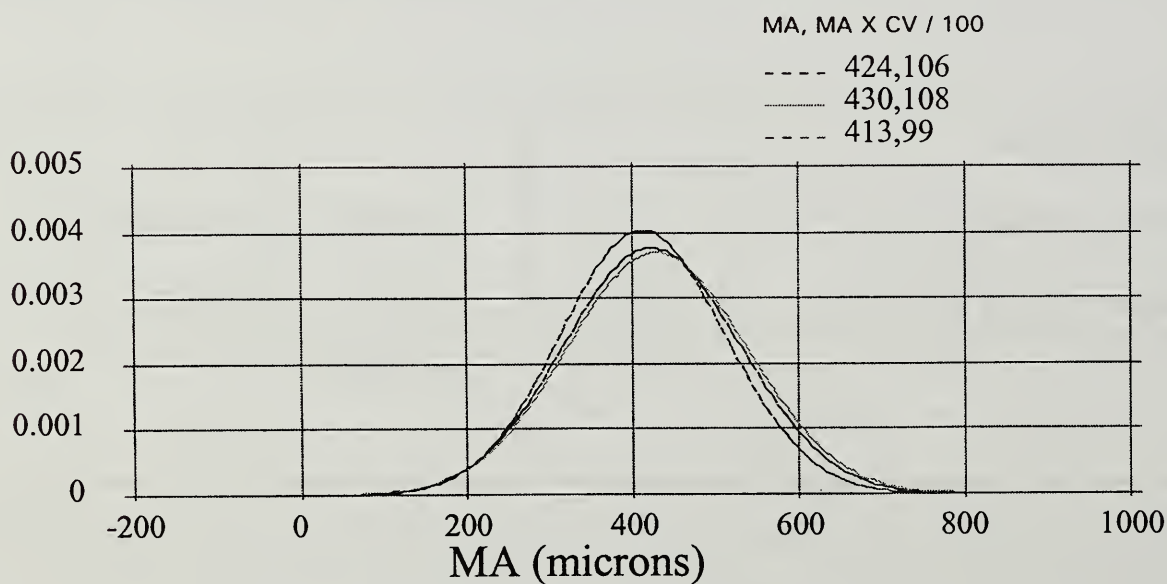


Figure 3. Effect of our acceptable tolerances on particle size distribution of fine sugar.

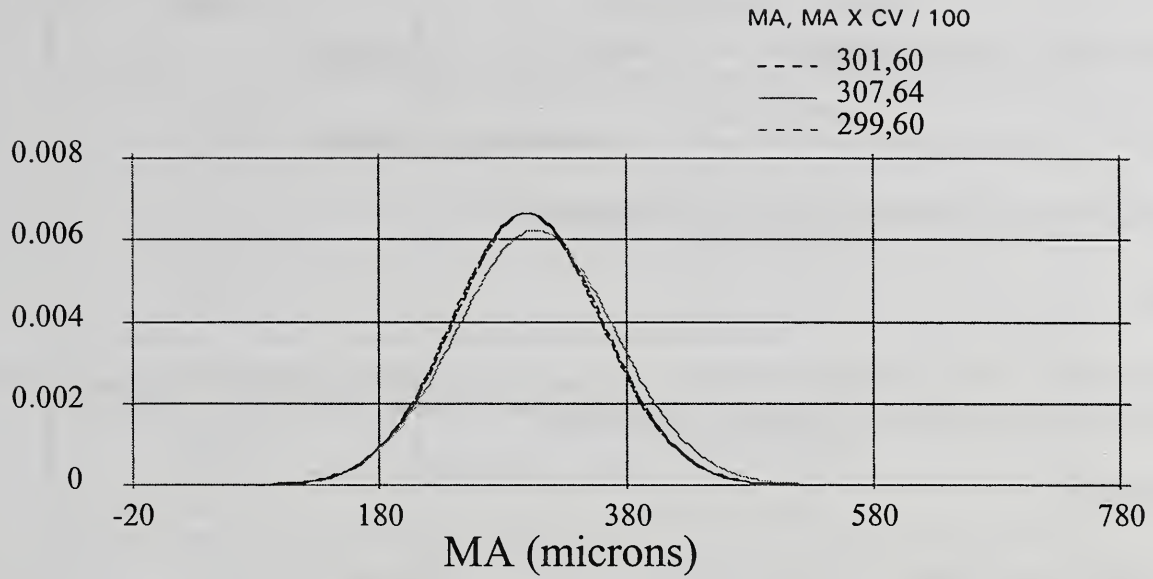


Figure 4. Effect of our tolerances on particle size distribution of extra-fine sugar.

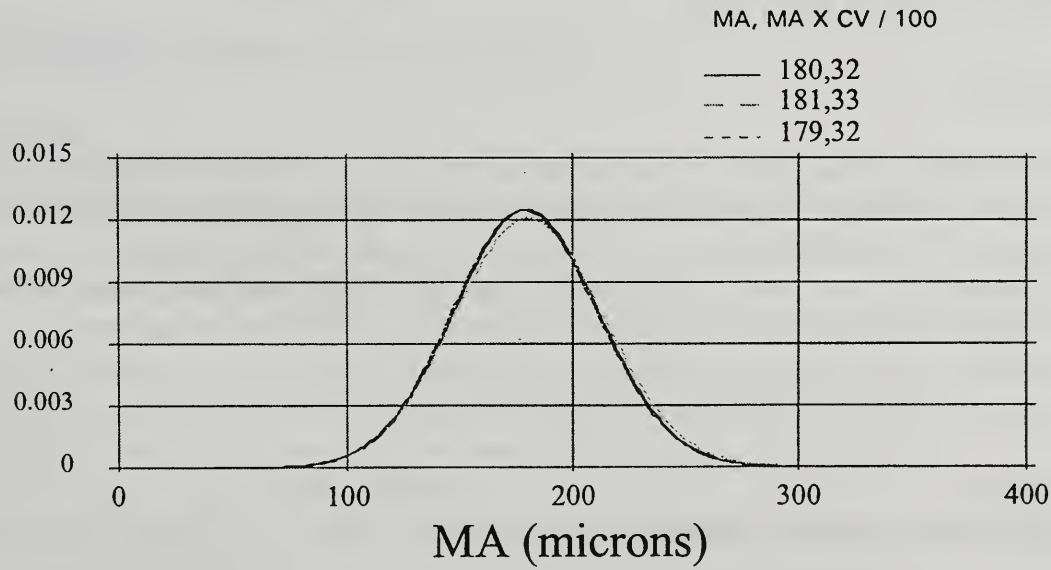


Figure 5. Effect of our tolerances on particle size distribution of powder sugar.

APPENDIX A. Copy of the Screen Verification Work Instruction

Sector: **QUALITY ASSURANCE**

Doc No.: **S-P-I-012**

Page: 1 of 4

Issue Date: **09/11/96**

Title: VERIFICATION OF SCREENING SIEVES

PURPOSE:

The purpose of this work instruction is to describe the method and frequency of calibration for screening sieves used for determination of granulated particle size.

REFERENCES:

Sieve Verification Data Sheet	S-P-F-157
Equipment Calibration Record Form	S-P-F-002
Master Calibration List	S-P-F-001
Procedure Manual, Section 11.0	S-P-PR-003
Certification Letters from W.S. Tyler	
ICUMSA Methods Book (1994) - Section GS2-37	
Laboratory Procedures Manual	S-Q-M-002
Master "Traceable or Certified Items" List	W-Q-F-001

RESPONSIBILITY:

It is the responsibility of the laboratory technician to calibrate the grist sieves at the prescribed frequency. The Quality Assurance Manager triggers the calibration by notifying the appropriate person at least two weeks in advance that the calibration is due. If the sieves are not within acceptable tolerances, they are discarded and replaced with new ones. The Quality Assurance Manager is responsible for maintaining calibration records and ensuring that the general procedures for calibration are adhered to.

METHOD:

Equipment Test Sieves	Location: Main Lab
Model # Canadian/U.S.A. Standard Testing Sieves	
Serial # Various	

Acceptable Tolerance:

Sieve size	Acceptable % variance (certified vs operating)
12 U.S.	-2.0 to +9.0%
16 U.S.	-3.0 to +7.0%
20 U.S.	-4.0 to +6.0%
30 U.S.	-4.0 to +6.0%
40 U.S.	-4.0 to +6.0%
50 U.S.	+2.0 to +12%
60 U.S.	0.0 to +12%
70 U.S.	-2.0 to +8.0%
80 U.S.	-4.0 to +6.0%
100 U.S.	-1.0 to +11%
200 U.S.	-4.0 to +6.0%

The screening sieves are calibrated every three months.

For each calibration:

1. Appropriate records are maintained (Ref S-P-F-002)
2. The calibration label is updated (Ref W-Q-F-001)

Each individual operating sieve screen is calibrated against a certified sieve traceable to national standards. If visibly damaged, it will be replaced at that time. Certified screens are used for calibration purposes only and are sent to the manufacturer every three years for re-calibration.

The following table summarizes the sugar types and scalping screens to be used for evaluation checks.

Screening sieves (U.S. std no.)	Sugar type	Scalping sieves
12	Coarse	None
16	Coarse	12
20	Coarse	16
30	Sanding	20
40	Sanding	30
50	Fine	40
60	Extra-fine	50
70	Extra-fine	50
80	Extra-fine	70
100	Powder	80
200	Powder	80

Screen analyses are done as per ICUMSA (1994) methods, GS2-37.

STEPS

1. Each screen is checked individually using the appropriate sugar and scalping screen for the corresponding U.S. Std sieve.
2. The percent weight is determined on the certified screen using the method as described above.
3. The sugar is recovered and the percent weight is determined on the operating screen of the same size. Note: scalping screen must be the same for both tests.
4. A comparison of the results is made and the percent error is calculated. (Note: certified screens are used as correct figure in the calculation).
5. If results are within the acceptable tolerance, the screens are accepted and recorded as such. If results are beyond acceptable tolerances, screens are discarded and results are recorded as such.

If during calibration checks, screens are identified as out of calibration, the Quality Assurance Manager must be notified and all products analyzed since the last good calibration must be questioned and addressed.

APPENDIX B. Validation Data SheetSector: **QUALITY ASSURANCE**Doc No.: **S-P-F-157**

Page: 1 of 1

Issue Date: **09/11/96**Title: **SIEVE VERIFICATION DATA SHEET:**

1	Sieve Serial No.	40-10	U.S. Standard Sieve Number	40
2	Tolerance			-4 to +6%

3	Scalping Sieve	1.4	Certified sieve result	+ 55.5
4	Scalping Sieve	1.3	In use sieve	- 55.1
5	Difference			= 0.4
6	Difference in Percentage	Line 5 / Line 3 in Percentage		0.7%
7	Sieve Accepted	If result on line 5 is lower or equal to tolerance on line 2		Accepted

8	Certified Sieve Number	2-19737
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By: **John Smith**Title: **Lab. Technician**Date: **02/13/98**
Month / Day / YearNext verification: **05/98**
Month / Year

POSTER

IMPROVED QUANTIFICATION OF SUCROSE, GLUCOSE AND FRUCTOSE IN MULTIPLE INDUSTRIAL SUGAR SAMPLES USING ION CHROMATOGRAPHY

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ABSTRACT

Ion chromatography with integrated pulsed amperometric detection (IC-IPAD) is gaining wide acceptance as the preferred technique for analyzing sucrose, glucose and fructose in industrial sugar samples, including juices and liquors. This article describes the further optimization of IC-IPAD to more accurately and repeatedly measure the amounts of sucrose, glucose and fructose in multiple industrial sugar samples. Using either a 16 to 160mM NaOH eluent gradient or a 100mM NaOH isocratic eluent method, the integration of peak areas, internal standard calibration with forced zero, linear calibration curves are shown to be the most accurate methods of quantitation. Check standards should be used to check the calibration accuracy. Calibration standard concentration effects were found to contribute to the less accurate quantification of oligosaccharides compared to monosaccharides, and correct concentration ranges are described. For gradient or isocratic IC-IPAD chromatographic runs, end-run washing with strong alkali eluent (for example, 200mM NaOH) was found to improve quantitation, and is necessary to remove strongly adsorbed anions. Five sugars were evaluated for their suitability as internal standards and both glucosamine and lactose were acceptable. Troubleshooting of problems that sometimes occur is discussed. Guidelines for improved quantitation of sugars are listed.

INTRODUCTION

Ion chromatography with integrated pulsed amperometric detection IC-IPAD (also known as high performance anion-exchange chromatography HPAE-PAD) is a liquid chromatography technique that is gaining wide acceptance as the preferred technique for the analysis of sucrose, glucose and fructose, as well as other simple sugars and oligosaccharides, in sugar industry samples. Such samples include juices, liquors and molasses from cane and beet sugar factories/refineries (1,2,3,4). Eggleston and Clarke (5) recently comprehensively described applications of IC-IPAD in the sugar industry. Separation on anion-exchange columns is achieved by exploiting the weakly acidic properties of sugars in alkaline conditions. Sugars are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. The technique is simple and direct (unlike gas chromatography, no derivatization is required), highly sensitive, generally accurate, specific and compatible with gradient elution techniques. IC-IPAD is presently being applied to a variety of routine monitoring and research applications, and official methods for sugar analyses in molasses samples, based on this technique, have also now been approved (6,7).

Many factories and refineries are dealing with multiple and varied samples, with a high throughput. In multiple sample analyses, some researchers use the bracketing technique of Thompson (8) where a

sample is sandwiched or bracketed between identical standards, to take account of any detector drift that may occur throughout the duration of the analysis. However, although chromatography software/integrators can be used to measure peak areas or heights, more time consuming manual calculations to obtain peak concentrations are required using the bracketing technique. In contrast, we use a technique in which a seven level calibration curve is first generated and concentrations can be automatically calculated using software. The use of check standards can overcome any detector drift, as well as quickly indicate the present accuracy of the column and methodology. Manual calculations are, therefore, minimized. Internal standard quantitation is widely used for IC-IPAD sugar analyses, as the detector response for each sugar component is different, and because errors caused by sample dilution, detector sensitivity, and changes in injection volume are minimized.

However, even though retention time precision is usually very good, quantitative accuracy and precision of simple sugar concentrations, especially sucrose, are sometimes disappointing. Furthermore, studies in this laboratory on the measurement of small sugar losses across industrial sugar processes or in model sugar degradation reactions, have necessitated the need for very accurate and absolute values of sugar concentrations. This prompted an in-depth study of the cause of the problems and how they can be improved. This paper describes some of the results, and discusses troubleshooting of problems which occur and lists guidelines drawn from some results of the study.

EXPERIMENTAL

Chemicals and Reagents

HPLC grade sodium hydroxide (50% solution) was obtained from Fisher Scientific. Millipore water (resistance of 18 M Ω) was used to prepare eluents and samples. Prepared eluents were sparged with high purity helium to remove carbonate.

Standards

All sugar standards were analytical grade. Fructose, raffinose and stachyose were obtained from Aldrich, sucrose, glucosamine-HCl, methyl- α -D-glucopyranoside and L-fucose from Sigma, glucose from J.T. Baker, myo-inositol from Calbiochem-Behring, and leucrose from Pfeifer and Langen.

IC-IPAD Chromatography Equipment

Chromatography System:	Dionex (Sunnyvale, CA) BioLC instrument
Pumps:	Dionex Gradient Pump Module (GPM)
Detector:	Dionex Model PED-2

S.P.R.I.

Detector Settings:	Working electrode pulse potentials and durations: $E_1=+0.05$ V ($t_0=0.00$ s), $E_2=0.05$ V ($t_1=0.42$ s), $E_3=+0.75$ V ($t_3=0.43$ s), $E_4=+0.75$ V ($t_4=0.60$ s), $E_5=-0.60$ V ($t_5=0.61$ s), $E_6=-0.60$ V ($t_6=0.96$ s). The duration of the IPAD integration interval was set at 0.2-0.4 s.
Cell:	Dionex gold (Au) working electrode and Ag/AgCl reference electrode
Autosampler:	Spectra-Physics SP8880 autoinjector, refrigerated at 4 °C
Columns:	Dionex CarboPac-1™ analytical (250 x 4 mm) and guard (25 x 4 mm) columns. A Dionex anion trap (ATC-1™) column was placed in the eluent line prior to the injection valve
Peak integration and quantitative calculations:	Dionex PeakNet Chromatography Workstation, version 4.3 (software)

IC-PAD Analysis of Sucrose, Glucose and Fructose: Isocratic Method

Flow rate was 1.0 mL/min at ambient temperature (~25 °C) and volume injected was 25 µl. Column eluent conditions were: 100 mM NaOH isocratic (0.0 to 20.0 min; inject 0.1 min). If an end-wash of strong alkali is included : 200 mM NaOH isocratic (20.0 to 23.0 min) and return to 100 mM NaOH (23.1 to 26.0 min) to re-equilibrate the column with the initial eluent prior to the next sample injection.

IC-IPAD Analysis of Sucrose, Glucose and Fructose: Gradient Method

Flow rate was 1.0 mL/min at ambient temperature (~25 °C) and volume injected was 25 µl. Column eluent conditions were: 16 mM NaOH isocratic (0.0-2.0 min; inject 1.0 min), a gradient of 16-160 mM NaOH (2.0-35.0 min), followed by isocratic 200 mM NaOH (35.1-40.0 min), and return to 16 mM NaOH (40.0-45.0 min) to re-equilibrate the column.

Preparation of Reference Calibration Standard Solutions

Unless otherwise stated in the text, it was found that the ranges of component sugars in seven levels of reference calibration standards (R) shown in Table 1 give valid, linear calibration curves and acceptable results.

IC-IPAD Operation

Using a Spectra-Physics SP8880 autosampler and Dionex Peaknet™ chromatography software, runs were accumulated of multiple standards and samples. First, a blank (B) was run to stabilize column and system performance, and then seven different levels (see Table 1) of the standards (R) were run, and standard curves were generated to test linearity and generate peak area response factors.

When the research objective was to test the repeatability or precision of a method the injection scheme was as follows:

Injection scheme (A) to test for repeatability:

B → R1 → R2 → R3 → R4 → R5 → R6 → R7 → R4 → R4 → R4 → R4 → R4 → R4 → R4 → R4 → R4 → R4 → B

When the research objective was to test how accurate the method was at determining amounts of component sugars in samples, which lie within the range of the calibration curve, the injection scheme was as follows:

Injection scheme (B) to test for accuracy:

B → R1 → R2 → R3 → R4 → R5 → R6 → R7 → R2 → R4 → R6 → B

Response factors were generated for each of the component sugars, using the internal standard calibration method. A forced zero was applied to the linear calibration curve as this had previously shown to improve accuracy.

Industrial Sample Analysis

Weight diluted industrial samples should be first filtered through a 0.45µm filter, then run in duplicate (i.e., S_A and S_B). The following injection scheme is used to run multiple samples of industrial samples:

Injection scheme to analyse multiple industrial samples:

B → R1 → R2 → R3 → R4 → R5 → R6 → R7 → R4 → S1_A → S1_B → S2_A → S2_B → R4 → S3_A → S3_B → S4_A → S4_B → R4 → etc..... → B

Response factors are generated for each of the component sugars, using the internal standard calibration method, and these are monitored using the R4 check standards.

RESULTS AND DISCUSSION

Routine monitoring of glucose, fructose and sucrose in industrial sugar samples can be undertaken using only a single run in less than 20.0 min, under isocratic NaOH eluent conditions. However, for accurate quantification of these sugars in industrial samples, it is necessary to undertake two runs: one to quantify

sucrose and another for invert sugars. This is because glucose and fructose (invert sugars) occur at much lower concentrations in industry samples and, consequently, the samples require less dilution. Unfortunately, this leaves the sucrose "overloaded" on the column and impossible to quantify, hence a separate run is required. For research purposes a gradient method using a longer run time (with more time for column washing and re-equilibration at the end of the run), is generally more accurate.

The following results and discussion highlight some precautions and improved methods which can contribute to the overall improved accuracy of quantitation of glucose, fructose and sucrose in industrial sugar samples, using IC-IPAD.

Sample Preparation

If samples are to be stored before analyses, they should be stored in a -40 °C freezer to prevent unwanted sugar degradation reactions from occurring. If a -40 °C freezer is not available then a -20 °C freezer will suffice, but storage time should be limited. A refrigerated (4 °C) autosampler will also ensure that degradation of sugars does not occur in samples in queue for analysis during of the multiple sample runs. Other workers (9) advocate the addition of small amounts of sodium azide to samples to prevent microbial induced breakdown of sugars. However, storing the samples in a -40 °C freezer and the use of a refrigerated autosampler has allowed us to eliminate this addition.

Quantification of Oligosaccharides Compared to Monosaccharides: Correct Linear Calibration Ranges

It was often observed in this laboratory that quantification of sucrose and other oligosaccharides (including raffinose and stachyose) was consistently more difficult, and less accurate, than for glucose and fructose (invert). Because sucrose, and to a lesser extent raffinose, occur in greater amounts than invert in industrial sugar samples, the range of their calibration curve standards had been higher, and concentration ranges that had been used are listed in Table 2. Check standards were used to test for accuracy, and precision or repeatability (see Experimental Section for full procedures) of this calibration method, and results are listed in Table 3. Average % RSD (relative standard deviation) for sucrose (3.69) was markedly higher than for glucose (1.38) and fructose (1.44), and % deviations from expected values of check standards were much higher. Other tests also showed unacceptable average % RSD values for sucrose of up to 6.23 and higher.

To test if this discrepancy in quantitation of oligosaccharides compared to monosaccharides was because of the differing concentration ranges in the calibration curve standards, more calibration standards were prepared with all sugar components having the same range as the monosaccharides, i.e., 1 to 7 ppm. These are listed in Table 4. Again check standards were used to test for accuracy and repeatability of this calibration method, and results are also shown in Table 3.

Although average % RSD for sucrose was improved from 3.69 to 2.45, this value could still be improved upon, because over the first part of the sucrose calibration curve (1 to 5 ppm) the response was not linear

but quadratic, and this is reflected in the lower R^2 value for the linear curve (see Table 3). This phenomenon was even more exaggerated for raffinose and stachyose, and is because they were too dilute to give valid IC-IPAD responses. Calibration standard concentration ranges for oligosaccharides, including sucrose, were consequently increased and final acceptable and working values are listed in Table 1.

Sucrose, with a range of 1 to 13 ppm, was shown to have a RSD of 1.66 (see Table 3) and gives consistently improved results. 1 to 13 ppm is equivalent to 25 to 325 ng in 25 μ l injections. LaCourse and Johnson (10) observed that glucose, fructose and sucrose oxidize differently at the gold (Au) working electrode and, consequently, exhibit different linear response ranges. Glucose, which is under transport controlled oxidation, produced a linear response over a larger concentration range than sucrose, which is under surface controlled oxidation (10). Fructose is under mixed controlled oxidation and exhibits intermediate linear response behavior between glucose and sucrose (10). LaCourse and Johnson (10) reported linear response behavior for sucrose up to \sim 34 ppm or 855 ng in 25 μ l injections, although this was estimated from only two calibration points on a current versus concentration plot and could, therefore, easily be lower. Shaffler *et al.*, (9) reported using only three levels of sucrose calibration standards ranging from 1000 to 1500 ng, using 20 μ l injections, to test for linearity. Others (11) have reported linear ranges for glucose and fructose of up to 1000 ppm. Results from this laboratory have not shown linearity at such high levels

Internal Standard Calibration: Which Internal Standard?

An investigation was undertaken to determine if different internal standards would improve quantitation accuracy and precision. A favorable internal standard should exhibit chromatographic properties similar to the analytes and should be well resolved from the nearest analyte, producing sharp peaks. Also, ideally, the amount of the internal standard should be slightly larger than the analyte peaks of interest (and the highest calibration standard level), but not be more than 10-fold higher (12). Furthermore, it should preferably be oxidized similarly at the gold working electrode. Glucosamine, a reducing sugar, has been the internal standard of choice at SRRC. Other workers (8,9,11) and the official IC-IPAD sugar methods (6,7) advocate lactose as the internal standard. However, lactose is a disaccharide reducing sugar and, therefore, may react differently at the gold electrode than the monosaccharide, glucosamine.

A series of commercially available sugars were investigated for their suitability as internal standards, including glucosamine and lactose. The internal standards were tested using the gradient IC-IPAD method. Both injection schemes (A) and (B) were run to test repeatability and accuracy, respectively, and results are listed in Table 5 and further illustrated in Fig. 1. Although Thielecke (13) found leucrose to be suitable as an internal standard, it was observed to co-elute with sucrose. Methyl- α -D-glucopyranoside (non-reducing) eluted too near the void volume to be viable. L-fucose was studied because it is widely (14) accepted as an IC-IPAD internal standard in the wood and pulp industry. However, although L-fucose showed good resolution, it is relatively expensive and lactose and glucosamine performed better (see Table 5). Although there were slight differences between the performances of lactose and glucosamine, they were generally the same. Both lactose and glucosamine would be valid internal standards.

End-Run Washing of Column with Strong Alkali

The use of an end-run wash with strong alkali (200 mM NaOH) is presently not advocated in the official IC-IPAD methods (6,7) for sugar analysis. However, at SRRC we have consistently used end-run washing to remove strongly adsorbed anions and, therefore, enhance column performance and prolong column life. Experiments were conducted to evaluate the effect of end-run washing on quantitation of glucose, fructose and sucrose, using the isocratic method with glucosamine as the internal standard. Results are shown in Table 6. When there was no wash, % deviations from expected values for the three levels of check standards were markedly higher and, therefore, more inaccurate. Moreover, average % relative standard deviations (RSD) were also higher, especially for sucrose, when no wash was used. These results strongly suggest that the incorporation of an end-run wash of strong alkali improves quantitation.

It was interesting to observe that even with a wash, the % RSD for glucose, fructose and sucrose in the isocratic method were obviously higher than for the gradient method with a wash, using the different internal standards (see Tables 3 and 5). Furthermore, the R^2 values for the calibration curves were significantly lower. This is likely because the separation of peaks, especially glucose and fructose, is much better when a gradient method is used. This confirms that the gradient method is generally more accurate than the isocratic method, but the disadvantage is the longer run time.

It must be noted here that when using an end-wash of strong alkali, it is important to re-equilibrate the column with initial eluent, to prepare the column for the next injection. This can be done at the end of the run or at the beginning of the next run, prior to injection

CONCLUSIONS

The improved procedures and methods described in this paper have contributed to the improved quantitative accuracy and repeatability of sugar concentrations in multiple industrial samples. This is particularly true when very accurate data are required for calculations of sugar losses across unit processes. From this research, a working calibration method and injection scheme to analyze multiple industrial samples are described in the Experimental Section. The use of check standards has been shown to be a valuable technique in which to determine the accuracy and repeatability of a calibration method and injection scheme. IC-IPAD quantitation methods are still being evolved, and further work is now required, using check standards, to evaluate and compare the bracketing, quantitation technique of Thompson (8). Also, the effect of electrode temperature control (9) on quantitation improvement needs to be investigated.

Troubleshooting tips and guidelines for the improved quantitation of simple sugars are given in Appendices A and B.

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Appendix A. Troubleshooting Tips for IC-IPAD

With IC-IPAD, as with any liquid chromatography technique, one must be aware of potential problems. It is extremely important to minimize contamination of the NaOH eluent solutions with carbonate (14). It is suggested that the following points be adhered to for the optimum operation of this technique for the analysis of multiple industrial sugar samples:

- ▶ Because of the complex mixture of colored and non-colored compounds present in industrial sugar processing samples (which includes salts, organic acids, polysaccharides etc.), guard columns and column frits frequently become fouled. Frits, especially at the inlet side of the guard column, should be inspected and replaced frequently. Guard columns usually need to be replaced less frequently. (Note: Frequency of replacement will depend on the number and frequency of samples that are analysed).
- ▶ The use of an anion trap column (for example, ATC-1™, Dionex Corp) used in the eluent line prior to the injection valve, helps to remove any trace amounts of carbonate from the eluent mobile phase. (Note: The ATC-1™ column cannot be used with sodium acetate eluent).
- ▶ Frequent monitoring of the gold (Au) working electrode is highly recommended. The use of a hand magnifying glass allows for careful inspection of the electrode. Electrodes with numerous dark pits should be replaced. Frequent cleaning of the electrode should be undertaken with a rubber eraser. The action of the eraser will form surface scratches on the electrode, but these do not adversely affect performance. However, a full polish with cleaning compounds is very harsh and reduces the life of the electrode and, therefore, should be undertaken as infrequently as possible.
- ▶ When multiple standards are run, a refrigerated autosampler (4° C) is recommended, to minimize the degradation of sugars in later samples, in queue for analysis.
- ▶ When multiple standards (more than three levels of standards are necessary for acceptable accuracy) and samples are run, using an autosampler, it is imperative that check standards are used, as column and temperature changes may affect the calibration. Standards should be first run to generate a calibration curve, then subsequent samples run with check standards. R^2 values of the generated internal calibration curves are not always a sufficient gauge of accurate quantification. If the curve intercepts both the y and x axis away from the zero point, then quantification could be over- or underestimates.

Appendix B. Guidelines for Improved IC-IPAD Quantitation of Sugars

1. Greater than 3 levels of calibration standards are required.
2. Calculate the peak area, not the peak height.
3. Use internal calibration, linear fit and forced zero calibration curves.

Standards linear range:

Sucrose: 1 to 13 ppm

Glucose: 1 to 7 ppm

Fructose: 1 to 7 ppm

4. Use glucosamine or lactose as internal standard.
5. Check standards are needed.
6. Glucose and fructose should be quantified in a different run to sucrose.
7. A wash of strong NaOH eluent should be run at the end of each chromatography run. Re-equilibration with the initial eluent must occur before the next injection.

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Table 1. Levels of component sugars in reference calibration standards (R).

Component sugars	Levels of component sugars in reference calibration standards (R) in PPM						
	R1	R2	R3	R4	R5	R6	R7
Myo-Inositol	1	2	3	4	5	6	7
Glucosamine (internal standard) ^a	15	15	15	15	15	15	15
Glucose	1	2	3	4	5	6	7
Fructose	1	2	3	4	5	6	7
Sucrose	1	3	5	7	9	11	13
Raffinose ^b	2	4	6	8	10	12	14
Stachyose ^c	4	8	12	16	20	24	28

^aOther internal standards were used as well - see text

^{b,c}Raffinose and stachyose were not component sugars in calibration standards used in the isocratic method

Table 2. Previous levels of component sugars in reference calibration standards (R).

Component sugars	Levels of component sugars in reference calibration standards (R) in PPM						
	R1	R2	R3	R4	R5	R6	R7
Myo-Inositol	1	2	3	4	5	6	7
Glucosamine (internal standard)	20	20	20	20	20	20	20
Glucose	1	2	3	4	5	6	7
Fructose ^a	2	4	6	8	10	12	14
Sucrose	1	5	9	13	17	21	25
Raffinose	2	4	6	8	10	12	14
Stachyose	4	8	12	16	20	24	28

^aFructose has a lower IC-IPAD response than glucose, consequently higher concentrations of fructose standards were first used

Table 3. Concentration effect of calibration standards on quantitation of glucose, fructose and sucrose.

Component sugar	Concentration range in calibration curve (ppm)	% deviation from expected values of 3 levels of check standards ^e	% rel std dev ^a of calculated amount	N	R ² (linear calibration curve)
Sucrose	1 to 25 ^b	-10.2 to 0 ^g	3.69 ^f	6	.999
	1 to 7 ^c	-4 to +3	2.45	6	.990
	1 to 13 ^d	-4.8 to +4	1.66	10	.997
Glucose	1 to 7 ^b	-4 to 0 ^g	1.38	6	.999
	1 to 7 ^c	-4 to -2	1.02	6	.998
	1 to 7 ^d	+2.7 to +4	1.14	10	.999
Fructose	2 to 14 ^b	-7.3 to -4 ^g	1.44	6	.999
	1 to 7 ^c	-6 to -1	1.96	6	.998
	1 to 7 ^d	0 to +4	1.01	10	.998

^a % RSD of up to 6.23 was also observed for sucrose^b See Table 2^c See Table 3^d See Table 1^e Using injection scheme (B) to test for accuracy - SEE EXPERIMENTAL SECTION^f Using injection scheme (A) to test for repeatability - SEE EXPERIMENTAL SECTION^g The following injection scheme was used: B → R1 → R2 → R3 → R4 → R5 → R6 → R7 → R3 → S1_A → S1_B → R3 → S2_A → S2_B → R3....etc....S6_A → S6_B → R3 → B

Table 4. Levels of component sugars in reference calibration standards (R) to check concentration effects.

Component sugars	Levels of component sugars in reference calibration standards (R) in PPM						
	R1	R2	R3	R4	R5	R6	R7
Myo-Inositol	1	2	3	4	5	6	7
Glucosamine (internal standard) ^a	10	10	10	10	10	10	10
Glucose	1	2	3	4	5	6	7
Fructose	1	2	3	4	5	6	7
Sucrose	1	2	3	4	5	6	7
Raffinose	1	2	3	4	5	6	7
Stachyose	1	2	3	4	5	6	7

Table 5. Characteristics of five different internal standards^a

Internal standard	R _t (min)	Resolution ^b (R)	% deviation from expected values of 3 levels of check standards ^c			% rel std dev ^d			N ^d	R ^{2d}			Comments
			sucr	glu	fru	sucr	glu	fru		sucr	glu	fru	
Leucrose	20.1	0.66	-	-	-	-	-	-	-	-	-	-	co-elutes with sucrose
Glucosamine	11.5	1.53	+4 to -4.8	+4 to +2.7	+4 to 0	1.66	1.14	1.01	10	.997	.999	.998	
Lactose	22.2	2.39	+9.5 to -11.7	+14 to +2	+6 to +2	0.45	0.34	1.74	6	.999	.997	.997	
Fucose	5.0	2.49	+5.6 to -6.6	+6 to -1	+6 to 0	0.68	0.4	2.80	10	.997	.997	.998	relatively high cost
Methyl-glucopyranoside	1.5	0.32	-	-	-	-	-	-	-	-	-	-	elutes too near void volume

^a 16 to 160mM NaOH gradient method with 200mM NaOH end-wash was used^b Resolution was calculated as the difference in retention of adjacent peaks divided by their average peak width^c Using injection scheme (B) to test for accuracy - SEE EXPERIMENTAL SECTION^d Using injection scheme (A) to test for repeatability - SEE EXPERIMENTAL SECTION

Table 6. Effect of end-run wash of strong alkali^a

Method type	% deviation from expected values of 3 levels of check standards ^b			% rel std dev ^c			N ^c	R ^{2c}		
	sucrose	glucose	fructose	sucrose	glucose	fructose		sucrose	glucose	fructose
Without wash	+3.3 to -3.6	+14 to -13	+12 to -8	6.16	1.39	3.17	10	.993	.987	.992
With wash	+5.3 to +1.7	+8 to -2	+6 to +2	0.76	1.1	3.66	10	.990	.992	.991

^a 100 mM NaOH isocratic method with or without 200mM NaOH end-wash was used - SEE EXPERIMENTAL SECTION^b Using injection scheme (B) to test for accuracy - SEE EXPERIMENTAL SECTION^c Using injection scheme (A) to test for repeatability - SEE EXPERIMENTAL SECTION

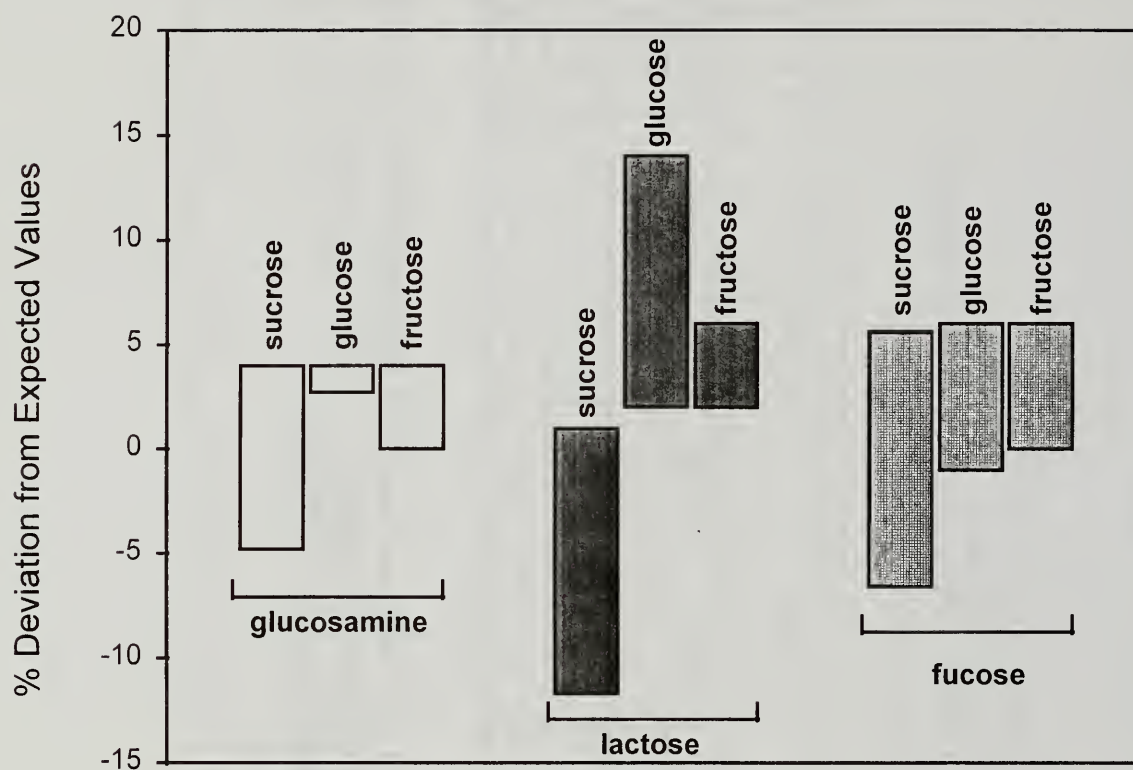


Figure 1. % deviation from expected values of three levels of check standards for glucosamine, lactose and fucose internal standards.

POSTER

IDENTIFICATION AND MEASUREMENT OF COMPOUNDS IN THE SURFACE FILM OF WHITE BEET SUGARS

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ABSTRACT

The tendency of a white sugar to form color on storage is an important element of quality. Previous studies at S.P.R.I. have examined the role of high molecular weight material occluded within the crystal on color development. Another source of potential color-forming reactions is the syrup coating on the crystal. In this study, trace components on the surface of representative white beet sugars from the United States and Europe were isolated, identified and measured. Compounds identified included organic acids, fatty acids, phenolic acids and pyroglutamic acid (pyrrolidone carboxylic acid). These compounds, present from the parts per billion to low parts per million range, arise both from the sugarbeet plant and from sugar degradation reactions.

INTRODUCTION

The major organic, non-sugar components of the beet plant and of beet processing are well documented (McGinnis, 1982). The concentration of important non-sugars in the sugarbeet plant is a significant parameter for beet breeders, and is also well documented (Smith, 1988). Less information is available on the presence of carbohydrate degradation products in beet sugar, or of phenolics, which are plentiful in the beet plant and contribute to color formation. Also, little data is available on the concentrations of organic acids in granulated sugar. Less information is available on the concentration of various organic and nitrogenous acids as they progress through the process, although their presence is well known.

Broughton and coworkers have studied the relationship of these compounds to color formation (Broughton, 1986, 1987; Shore, 1984) and have extended the work of Carruthers, et al. (1961) who noted that although many of the well known constituents of thick juice occur in similar proportions in white sugar and in the mother liquor, some components are present in relatively higher proportions in the granulated sugar than in the thick juice. They determined an elimination factor for important components, including: potassium (E.F.=131), sodium (E.F.=125), betaine (E.F. = 132), pyroglutamic acid (pyrrolidone carboxylic acid) (E.F.=113), glutamic acid (E.F.=200), raffinose (E.F.=20). The higher the E.F., the better the elimination.

Parts of this study were reported earlier (Godshall, 1997; Godshall and Clarke, 1997).

Properties of Minor Components/Color Precursors

Many of the minor components in beet processing have the potential to form color or to influence color formation, by such reactions as self-polymerization (as with, for example, hydroxy-methylfurfural), oxidation (as with, for example, polyphenolics) or to act as catalysts for color formation (organic acids, amino acids, reducing sugars). The organic non-sugars may be considered potential color precursors.

Some of the properties to consider when studying the non-sugars are:

Location in the crystal. As discussed above, color is classified as to whether it is inside the crystal or on the surface of the crystal. Its location may suggest its chemical nature.

Source. The major sources of non-sugars include the beet plant, microbial activity and sugar breakdown. Inclusion of beet crowns and tops increases the potential for non-sugars and colorant in the sugar. Some major constituents, such as lactic acid, a primary product of alkaline decomposition of sucrose and a major microbiological product, may have several sources. Other compounds, such as pyroglutamic acid and glutamic acid derive from precursors in the beet plant (ie, glutamine) and are produced in process depending on whether conditions are acid or alkaline.

Molecular weight. The molecular weight of the non-sugars ranges from polymeric (up to several hundred thousand daltons) through oligomeric, small molecules and volatiles (Clarke, 1989, 1991; Godshall, 1991, 1996). Polymeric and larger molecules tend to co-crystallize with sucrose and are found inside the crystal, especially if they are carbohydrate in nature or bound to carbohydrate. Raffinose and other carbohydrate oligomers also will preferentially go into the crystal, co-crystallizing with sucrose, and may even affect crystal shape. The small molecules are often on the surface. Volatiles are usually found on the surface.

Chemical Type. Chemical nature underlies all the properties of organic non-sugars. The chemical nature determines location in the crystal and whether color will develop over time (in storage) or under adverse environmental conditions. Chemical types found in sugar include polysaccharides, oligosaccharides, phenolics, organic or fatty acids, nitrogenous compounds, volatile fatty acids, and hydrocarbons.

Purpose of the study

As part of an ongoing study on the nature of sugar colorant at Sugar Processing Research Institute, Inc., we have long been interested in color development on storage (Clarke, *et al.*, 1991). Color increase on storage depends not only on external factors such as heat and moisture but also on the chemical nature of the impurities, both on the surface of the crystal and within the crystal. A significant proportion of the color increase may occur in the thin film of syrup that coats the crystal, as this layer presents a greater surface area exposed to air and oxygen, has a higher moisture content, and may contain the major proportion of the smaller, reactive molecules that carry over from the raw juice or that are formed on processing.

The amount of reported surface colorant in beet white sugar varies from approximately 20% of the total color to 54%. Shore, *et al.*, (1984) reported an average value of surface color of 20-25%; Broughton, *et al.*, (1988) reported an average of 43%, and Godshall, *et al.*, (1991) reported 54%. The range of surface color in the latter study was broad, from 15% to 88%.

The purpose of this study was to isolate, identify and measure compounds on the surface of the crystal that may contribute to color increase over time. A separate presentation will discuss changes that occurred during storage (Godshall, *et al.*, 1998)

MATERIALS AND METHODS

Seven granulated sugars, two from the United States and five from Europe, were quantitatively extracted and the minor constituents in the surface coating of the crystal were identified and measured by gas chromatography/mass spectrometry (GC/MS).

The compounds were extracted from the surface of the granulated sugar by an ethanol wash, performed by slowly stirring 100 g sugar with 120 ml ethanol in a 1-liter beaker with a large stir bar (2") for two hours at room temperature. The sugar was filtered on a coarse sintered glass funnel and the ethanol extract evaporated just to dryness. The residue was taken up in 10 ml water, acidified with 200 μ l 4N HCl, and re-extracted with three 15-ml aliquots of ethyl acetate. The ethyl acetate extract was dried over sodium sulfate for at least one hour, filtered and evaporated to dryness. The ethyl acetate extraction eliminated the excess of sucrose that was extracted by the ethanol in the first extraction. The large amount of sucrose present in the ethanol extract would have interfered with measurement of the trace quantities of the other components. The extracted components were converted to their volatile trimethylsilyl derivatives with Tri-Sil Concentrate. Trehalose was used as the internal standard.

Separation of constituents was accomplished using a Hewlett Packard Series II Model 5890A gas chromatograph coupled with a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and a 70eV electron impact ion source. The column used was 30m x 0.25cm with 0.25 μ m film thickness, 5% phenyl methyl silicone phase. Temperature program was 85°C for 4 min; increase 4°C per min to 100°C, then increase 10°C per min to 250°C for 10 min.

Compounds were identified on the basis of their mass fragmentation patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with a S.P.R.I. mass spectral library of standards.

RESULTS AND DISCUSSION

Table 1 shows the results of duplicate extractions on one sugar, indicating that the procedure was sufficiently reproducible for the low concentrations encountered.

Ethanol washings of the surface of white beet sugar crystals usually yielded a clear, pale to bright yellow solution, with a slightly fatty odor. The results of the extractions are summarized in Table 2, showing the different categories of compounds identified.

The major acids reported in raw juice by Oldfield, et al. (1974) were lactic, oxalic, phosphoric, malic, pyroglutamic (pyrrolidone carboxylic acid), and citric acid. In the study reported here, the major acids were lactic, glycolic, succinic, palmitic and stearic. Tartaric acid was a major acid in two sugars from the same European producer and absent in the others. Citric and oxalic acids were noted as trace components, too low to measure, and so are not listed in the table. Their relative absence may be due to their more-or-less complete removal as insoluble calcium salts during processing. Phosphoric acid is probably not very efficiently extracted by ethyl acetate.

The quantities found were low, in the parts-per-billion range. The sugars showed a considerable range in concentration and type of compounds found. Not reported previously is the relatively high level and number of fatty acids and alcohols.

Figure 1 shows a portion of the chromatogram of Sugar D. This constellation of peaks is typical of a white beet sugar surface extract, and includes glycerol (retention time 6.79 min), phosphoric acid (trace at 6.85 min), unknown (7.11 min), unknown m/z 145 (7.27 min), succinic acid (7.51 min), unknown, possibly 2-methyl-2,3-dihydroxy-propanoic acid (7.75 min), and glyceric acid (8.00 min).

The peak at 7.27 min is of interest because it is a major component in many sugars, which we have not been able to identify. Its spectrum is shown in Figure 2. The spectrum is characterized by a prominent mass at m/z 145. It appears to be five-carbon acid or alcohol. Five-carbon compounds such as 2-hydroxy-valeric acid, 2-hydroxy-3-methyl-butyric acid, and 2-hydroxy-2-methyl-butyric acid have prominent m/z 145 peaks in their mass spectra, but none of the authentic standards corresponded to our unknown peak. It could conceivably be a C-5 polyhydroxy alcohol, such as 2-methyl-1,2-butanediol or 2,3-didesoxypentitol, both of which have prominent m/z 145 peaks in their spectra, but we did not have samples of the authentic compounds to test as standards.

Sugar E is of interest, because it was nine years old, and is the sugar that was reported in Godshall, *et al.* (1991). At the time this sugar was received, its color was 60, a doubling of the 30 color it had when produced. The surface colorant was 73% of the total color. By the time of this study, color had increased to 99, and only 25% of the color was in the surface, indicating that more color had formed inside the crystal. At the time the sugar was received, we did not have the capability to identify the minor constituents by GC/MS, so we do not have baseline data on it.

The presence of fatty acids is quite remarkable. One sugar (not listed in the table) was characterized by a series of saturated fatty acids, mostly in trace quantities, including C8, C9, C10, C11, C12, C13, C14, C15, C16 (palmitic acid) and C18 (stearic acid). The latter two were major components and are present in almost all white sugar extracts. The series of fatty acids from C8 through C18 (with the exception of C11 and C12) was also noted in the surface extract of Sugar G.

Occasionally present in the ethanolic washings of these and other sugars were several phenolic derivatives: para-hydroxy-benzaldehyde, ortho-hydroxy-phenylacetic acid, 4-hydroxy-phenylacetic acid

ethyl ester, 4-hydroxy-benzeneacetic acid, and vanillic acid, as well as several unidentified branched and straight chain hydrocarbons, in the C15 to C22 range, and compounds that appeared to be steroids or sterols noted in trace quantities. Oleanolic acid, implicated in floc formation, was present as the free acid in two sugars.

There was no strong correlation of any of the components identified with the color of the sugars, except for lactic acid, which had a 0.89 correlation coefficient (Figure 3). The correlation coefficient of glyceric acid with color was 0.54 and with glycolic acid was 0.65.

There is little evidence of carbohydrate degradation compounds in these sugars. Two meta-saccharinic acid lactones, 3-deoxyarabino-hexonic-acid-gamma-lactone, and 2-methyl-ribonic-acid-gamma-lactone, were present in two of the sugars. Metasaccharinic acids are products of alkaline degradation of reducing sugars (Sowden, 1957). We found 3-deoxyarabino-hexonic-acid-gamma-lactone to be a major product of alkaline degradation of glucose. The structure of the latter compound is shown in Figure 4. The absence of HMF in these sugars is noted.

SUMMARY AND CONCLUSIONS

With only seven sugars, the sample size in this study was small, but it would appear that sugars with higher color tended to have a higher concentration over-all of components in the crystal surface. The diversity of compounds is notable, particularly the number of fatty compounds. There is no strong correlation of color with any one single component, the strongest being 0.89 with lactic acid. Geographic factors may also influence the presence of some compounds: for example, the tartaric acid present in two sugars from the same country in Europe and not found in the other five sugars.

Several carbohydrate degradation products, such as 3-deoxy-arabino-hexonic acid gamma lactone, a major degradation product of alkaline glucose degradation, were present. The absence of 5-hydroxymethyl-2-furfural in all the sugars tested was noted.

Most of the compounds reported in this study are common constituents of foodstuffs because they are common to most plants. The concentration of components was in the parts-per-billion range.

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Table 1. Duplicate extracts of a white beet sugar (sugar A).

Compound	Concentration, ppb on sugar	
	No. 1	No. 2
Lactic acid	1903	2320
Glycolic acid	204	230
Benzoic acid	16	17
Octanoic acid	6	7
Glycerol	27	45
Succinic acid	215	284
Glyceric acid	46	79
Fumaric acid	15	29
Nonanoic acid	20	23
Decanoic acid	4	14
Malic acid	67	88
L-pyroglutamic acid	921	1114
p-OH-Benzoic acid	34	32
Cetyl alcohol	21	11
Sorbitol	8	5
Palmitic acid	106	73
Linoleic acid	75	68
Stearic acid	83	50
Oleanolic acid	11	30

Table 2. Compounds identified on the surface of white beet sugars, reported as ppb on sugar.

Compound	Sugar A	Sugar B	Sugar C	Sugar D	Sugar E	Sugar F	Sugar G
Organic Acids							
Lactic Acid	1903	251	597	495	2770	678	180
Glycolic Acid	204	17	54	31	160	29	21
Succinic Acid	215	9	63	33	197	52	19
Glyceric Acid	46	9	28	8	38	10	10
Fumaric Acid	15	0	4	3	0	2	0
Malic Acid	67	0	42	26	17	11	4
Tartaric Acid	0	339	3210	0	0	0	0
Fatty Compounds							
Octanoic Acid	6	Trace	Trace	0	3	1	0
Nonanoic Acid	20	0	3	Trace	4	3	Trace
Cetyl-OH	21	31	53	17	9	8	28
Palmitic	106	17	250	53	56	44	49
Octadecan-1-ol	13	6	18	7	7	7	7
Linoleic Acid	75	3	34	0	0	0	0
Oleic Acid	Trace	3	14	18	19	5	6
Stearic Acid	83	6	106	64	69	57	45
Phenolics							
Benzoic Acid	16	6	0	Trace	6	2	Trace
p-OH-Benzoic	34	0	0	0	13	Trace	0
p-OH-Cinnamic	0	0	0	0	6	Trace	0
Nitrogenous							
Glutamic Acid	0	0	19	4	38	8	0

Table 2. (continued)

Compound	Sugar A	Sugar B	Sugar C	Sugar D	Sugar E	Sugar F	Sugar G
Pyrrolidone carboxylic acid	421	0	35	0	374	32	0
Carbohydrate Degradation							
3-deoxyarabino hexonic acid γ lactone	0	0	9	0	22	0	0
2-Me-ribonic acid γ lactone	0	0	7	0	36	3	0
Gluconic Acid	0	38	73	0	0	0	0
Miscellaneous							
Glycerol	27	18	34	16	12	11	11
Phosphoric Acid	0	0	0	0	12	7	0
m/z 145 (C5)	13	207	482	54	66	66	30
Xylitol	0	29	366	0	0	0	0
Oleanolic Acid	11	0	0	0	4	0	Trace
ICUMSA Color							
	42	10	18	39	99	33	26

Sugars E and F are from the United States.

Other sugars are from Europe.

Sugars B and C are from the same country and producer.

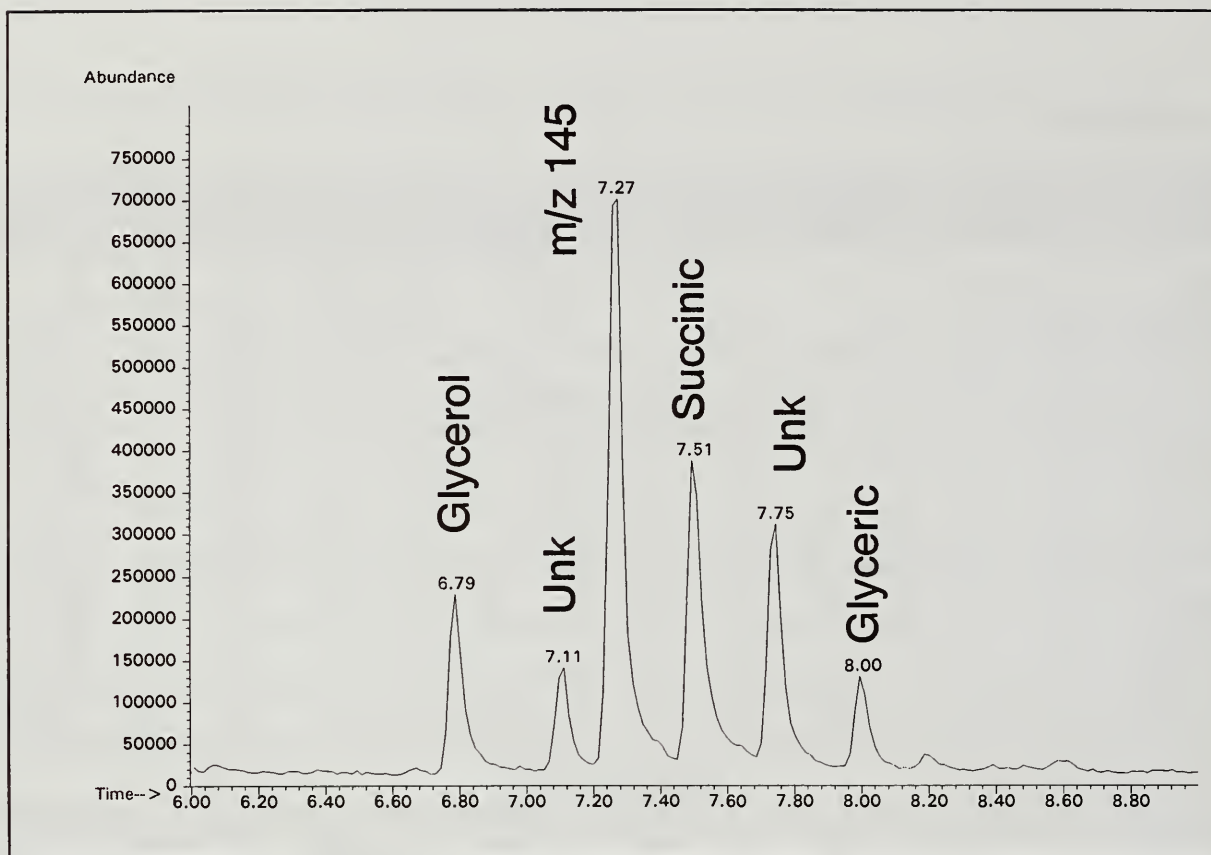


Figure 1. Peaks found in surface of white beet sugars.

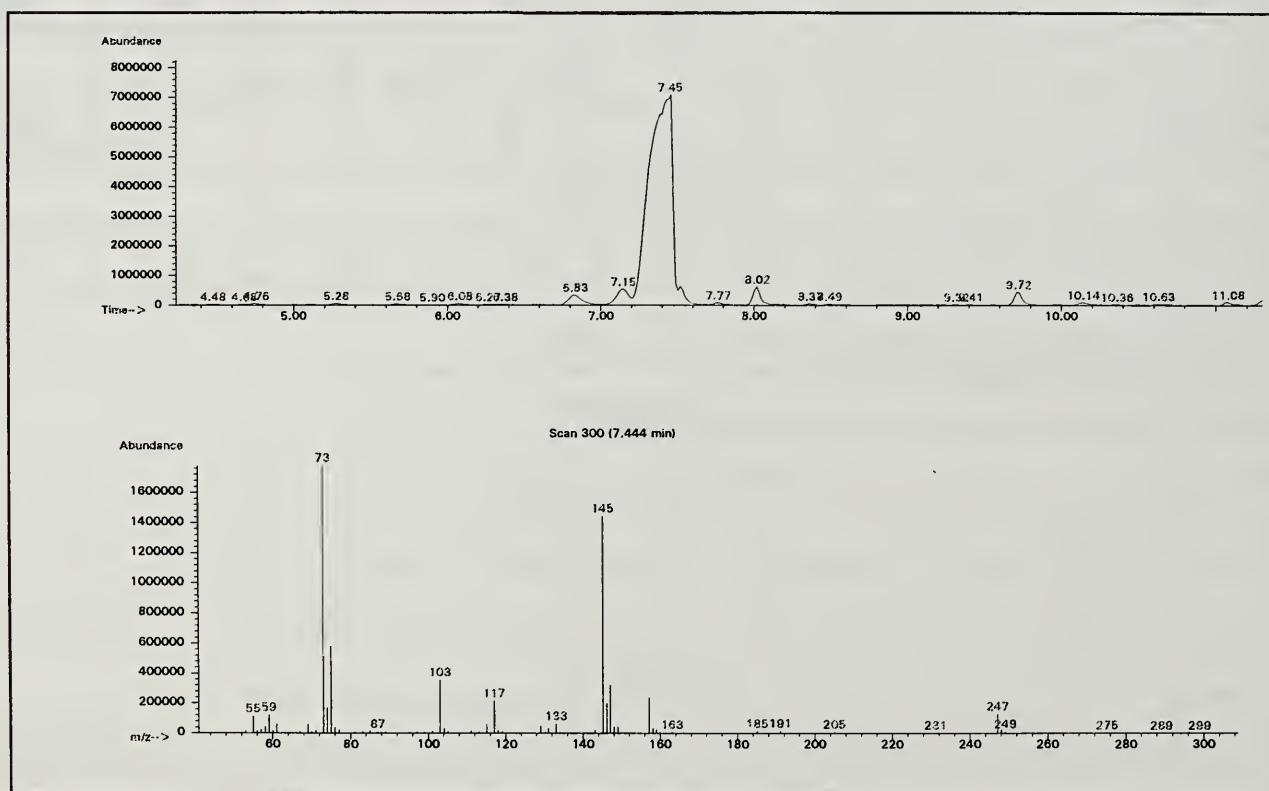


Figure 2. Unidentified 5-C acid, a major component in surface of white sugar.

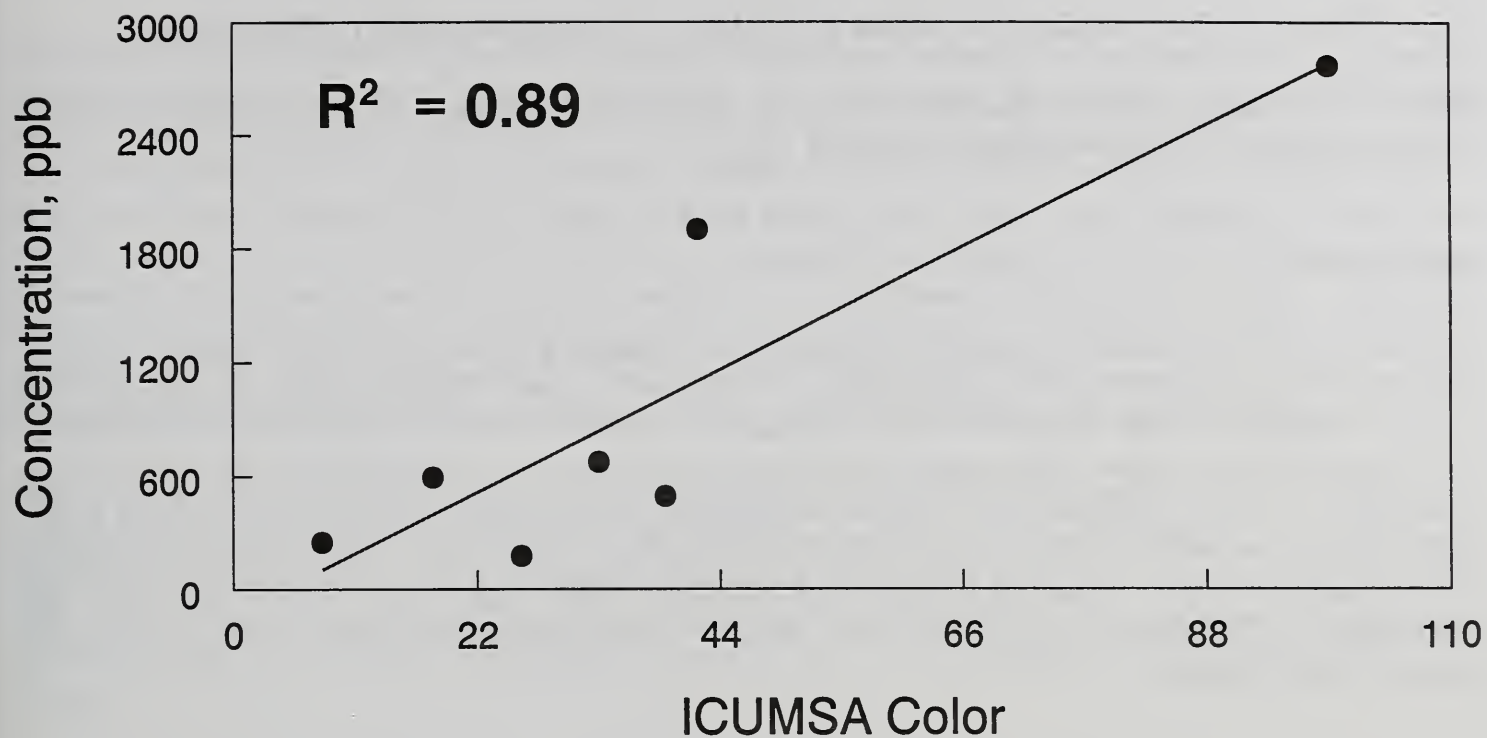


Figure 3. Correlation of color with lactic acid concentration.

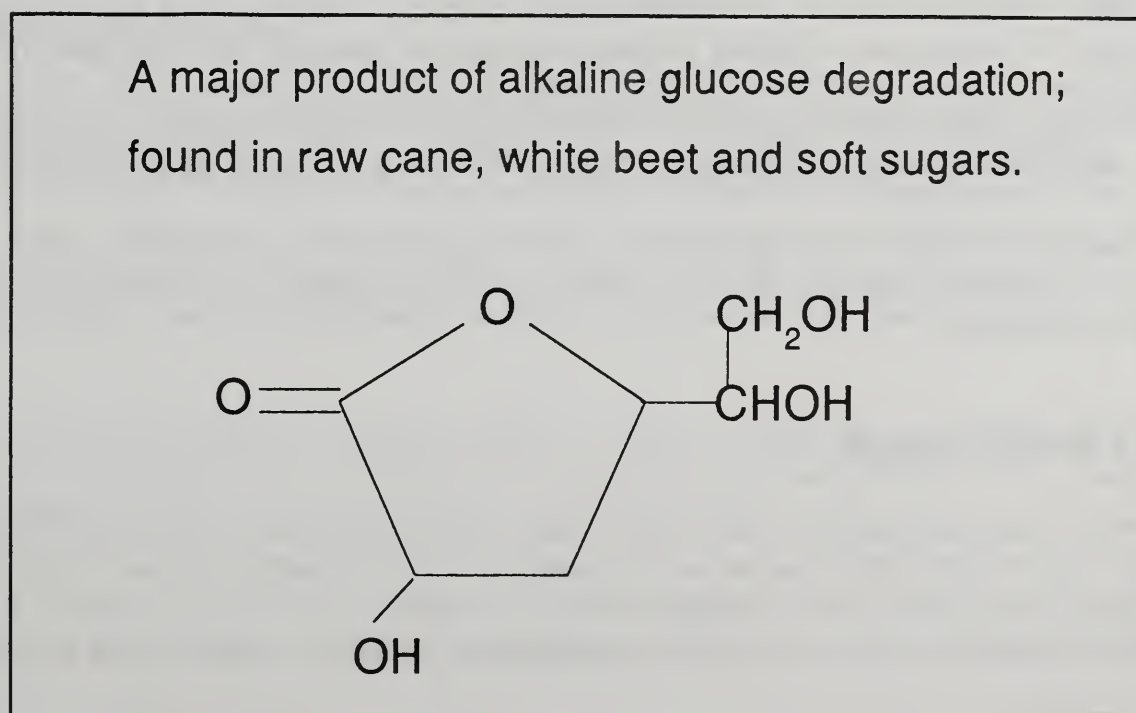


Figure 4. 3-deoxy-arabino-hexonic acid gamma lactone.

POSTER

CHANGES IN WHITE SUGARS UNDER ACCELERATED STORAGE CONDITIONS

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ABSTRACT

In an earlier study, a number of organic compounds, encompassing carboxylic acids, phenolic acids and fatty acids were identified on the surface film of white beet sugars. The formation of color and the changes in several of these constituents over time under accelerated storage conditions were examined in several beet and cane sugars. High quality white sugars were stable for an extended period of time when stored at 55°C. Lactic acid and 2-pyrrolidone-5-carboxylic acid (pyroglutamic acid) were present in the highest concentrations (0.5-2 ppm) and showed large changes in some sugars. These may be involved in color-forming reaction. With the exception of lactic acid and 2-pyrrolidone-5-carboxylic acid, the concentration of identified compounds did not exceed 0.5 parts-per-million and was often in the low parts-per-billion range.

INTRODUCTION

An earlier study at Sugar Processing Research Institute, Inc., had shown the presence of numerous organic compounds in the surface film of white beet sugar crystals (Godshall, *et al.*, 1998). It was felt that some of these compounds had the potential for involvement in color formation during storage of the sugar. The types of compounds included hexonic acid gamma lactones, phenolic acids, fatty acids, carboxylic acids, amino acids, and 2-pyrrolidone-5-carboxylic acid (pyroglutamic acid). The presence of this mixture of compounds in the outer, syrup layer of the crystal, in contact with moisture, air and light, spread over a thin surface, seemed to present many opportunities for continued color formation.

In this study, sugars of mostly beet origin, but some cane sugars as well, were stored under accelerated storage conditions and the change in color noted. Representative samples were extracted for observation of the change in compounds.

MATERIALS AND METHODS

Twenty-one white granulated sugars, 4 cane and 17 beet, were obtained from sponsoring companies of S.P.R.I. Their date of production was known so that the colors of freshly produced sugars could be obtained. Sugars were stored in glass stoppered bottles in an oven set to 55°C and removed at various weekly intervals to determine color, pH, turbidity and indicator value (I.V., ratio of color at pH 9 to color at pH 4).

Turbidity was measured as the difference in color before and after filtration on a 0.45 μ Millipore membrane during the ICUMSA color test, and is reported in ICUMSA color units (ICU).

Compounds on the surface of the crystal of selected sugars were extracted by an ethanol wash, performed by slowly stirring 100 g sugar with 120 ml ethanol in a 1-liter beaker with a large stir bar (2") for two hours at room temperature. The sugar was filtered on a coarse sintered glass funnel and the ethanol extract evaporated just to dryness. The residue was taken up in 10 ml water, acidified with 200 μ 4N HCl, and re-extracted with three 15-ml aliquots of ethyl acetate. The ethyl acetate extract was dried over sodium sulfate for at least one hour, filtered and evaporated to dryness. The ethyl acetate extraction eliminated the excess of sucrose that was extracted by the ethanol in the first extraction. The large amount of sucrose present in the ethanol extract would have interfered with measurement of the trace quantities of the other components. The extracted components were converted to their volatile trimethylsilyl derivatives with Tri-Sil Concentrate. Trehalose was used as the internal standard.

Separation of constituents was accomplished using a Hewlett Packard Series II Model 5890A gas chromatograph coupled with a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and a 70eV electron impact ion source. The column used was 30m x 0.25cm with 0.25 μ m film thickness, 5% phenyl methyl silicone phase. Temperature program was 85°C for 4 min; increase 4°C per min to 100°C, then increase 10°C per min to 250°C for 10 min.

Compounds were identified on the basis of their mass fragmentation patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with a S.P.R.I. mass spectral library of standards.

RESULTS AND DISCUSSION

Figure 1 shows the change in color that occurred in 20 of the sugars (3 cane and 17 beet) after 4 weeks (28 days) of storage at 55°C. The color of fresh sugars ranged from 4 to 39 ICU and the stored color ranged from 7 to 55 ICU. The color increase ranged from 0% to 91%. This represented an average color increase of 23%. Although the color nearly doubled for one sugar, none of the sugars reached what may be considered the maximum allowable white sugar color of 60 ICU. The color increased in all the sugars, but it remained within an acceptable range, and none of the sugars appeared degraded or visibly colored to the eye.

Table 1 summarizes the pH, color, turbidity and I.V. of the 20 sugars that are profiled in Figure 1. The data showed little change in the I.V., and only a small drop in pH and turbidity. The averaged data are summarized below:

Average color, fresh	26
Average color, stored	32
Maximum stored color	55
Average color increase	23%
Maximum color increase	91%
Average I.V., fresh	1.42
Average I.V., stored	1.41
Average pH, fresh	6.41
Average pH, stored	6.22
Average turbidity, fresh	12
Average turbidity, stored	10
Number of sugars in study	20

Table 2 shows the changes that occurred in three selected sugars (beet) that occurred over a storage period of 6 weeks. Sugar C showed a large increase in both lactic acid and pyroglutamic acid; Sugar G showed an increase in pyroglutamic acid. The one cane sugar that was extracted (data not shown) did not contain any pyroglutamic acid over the period of the study. It did contain lactic acid at about the 300 ppb range and also HMF(5-hydroxymethyl-2-furfural), which was not found in any of the beet sugars extracted in this study or in the previously mentioned study, possibly indicating different routes for degradation and color formation.

Table 3 shows in detail the results of the extraction of Sugar C. After being stored at elevated temperature for 6 weeks, this sugar had undergone a 46% increase in color. At 38 ICU, however, the stored color was still well within a safe range. Several compounds appeared to increase on storage, especially between 2 weeks and 6 weeks. Except for pyroglutamic acid, these were acids that could be products of carbohydrate degradation.

Pyroglutamic acid is a major nitrogenous compound in sugarbeet processing, arising from the glutamine in the beet plant. Under acidic conditions, glutamine will form glutamic acid and under alkaline conditions or heat, will lose ammonia to form pyroglutamic acid. Glutamic acid and pyroglutamic acid are in equilibrium with one another, glutamic acid dehydrating to form pyroglutamic acid and pyroglutamic acid rehydrating to form glutamic acid (McGinnis, 1982). This process is shown in Figure 2. It is possible that the increase in pyroglutamic acid is due to conversion of glutamic acid under storage conditions. Up to 38 ppb of glutamic acid has been observed in this study in beet sugars. Since glutamic acid is sparingly soluble in ethanol and ethyl acetate, it is possible that the extraction procedure is not effective for glutamic acid.

SUMMARY AND CONCLUSIONS

This study was an extension of the earlier work that showed the presence of beet-derived and process-derived compounds in the syrup layer on the surface of white beet sugars. It has shown that high quality (low color and moisture) beet sugars are quite stable to extended periods of storage at moderately high temperatures (55°C for 4-6 weeks).

Lactic acid and pyroglutamic acid are the major components identified and appear to change over time in some sugars, increasing as color increases. Other parameters, such as turbidity, pH and I.V., did not show a significant change, on average, over 4 weeks of storage. The I.V., which is the ratio of color at pH 9 to color at pH 4, is an indicator of the pH sensitivity of the color and an indication of the presence of plant derived phenolic-related colorant. It tends to go down in sugars that develop process-derived colors. The I.V. is typically low in beet sugars, in the range of 1.3-1.5.

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Table 1. Summary of color data for white sugars on storage.

Sugar	Baseline/Fresh				Stored 4 weeks @ 55° C			
	pH	Color	Turbidity	I.V.	pH	Color	Turbidity	I.V.
Beet-A (Europe)	6.22	4	4	1.66	5.92	7	1	2.17
Beet-B (Europe)	6.53	22	20	1.47	6.52	42	12	1.68
Beet-C (USA)	6.12	26	8	1.36	6.12	34	10	1.33
Beet-D (USA)	5.98	21	9	1.29	5.91	29	5	1.23
Beet-E (USA)	6.72	39	10	1.28	6.27	50	9	1.22
Beet-F (USA)	6.26	38	6	1.35	6.17	55	6	1.29
Beet-G (USA)	6.75	33	7	1.24	6.15	36	6	1.28
Cane-H (USA)	6.78	25	19	2.58	6.45	28	18	2.55
Cane-I (USA)	5.75	37	10	1.66	5.91	42	4	1.62
Beet-J (USA)	6.57	29	19	1.25	6.49	29	16	1.15
Beet-K (USA)	6.88	35	9	1.23	6.88	35	9	1.27
Beet-L (USA)	5.89	14	5	1.46	5.93	21	3	1.29
Beet-M (USA)	7.03	16	8	1.26	6.55	20	6	1.24
Beet-N (USA)	6.17	15	19	1.13	5.77	24	14	1.25
Beet-O (Europe)	5.44	23	16	1.35	4.98	22	17	1.44
Beet-P (Europe)	6.92	29	34	1.27	7.13	27	34	1.28
Beet-Q (Europe)	6.77	29	15	1.19	6.53	31	15	1.13
Beet-R (Europe)	6.94	36	14	1.21	6.68	42	11	1.24
Cane-S (USA)	6.35	33	5	1.93	6.05	37	3	1.22
Beet-T (Europe)	6.11	19	4	1.29	5.89	34	3	1.32

Table 2. Changes in 3 beet sugars on storage at 55°C.

Sugar C	ICU	Turbidity	I.V.	pH	Lactic acid	PCA*
Baseline	26	8	1.36	6.12	921	575
1 Week	28	6	1.67	6.09	830	586
2 Weeks	33	4	1.39	5.89	935	490
4 Weeks	34	10	1.33	6.12	1588	722
6 Weeks	38	4	1.23	5.63	2431	1146
Sugar G	ICU	Turbidity	I.V.	pH	Lactic Acid	PCA
Baseline	33	7	1.24	6.75	450	29
1 Week	41	13	1.36	6.53	760	30
2 Weeks	37	12	1.44	6.52	409	26
3 Weeks	39	14	1.38	6.79	520	63
4 Weeks	36	6	1.28	6.15	725	61
6 Weeks	41	13	1.24	6.66	869	59
Sugar J	ICU	Turbidity	I.V.	pH	Lactic acid	PCA
Baseline	29	19	1.25	6.57	844	208
1 Week	29	9	1.21	6.20	647	126
2 Weeks	33	9	1.24	6.18	359	152
3 Weeks	33	10	1.26	6.25	622	170
4 Weeks	29	16	1.15	6.49	843	188
6 Weeks	33	6	1.22	5.88	852	215

*PCA = 2-pyrrolidone-5-carboxylic acid (pyroglutamic acid)

Lactic acid and PCA are shown in ppb on sugar.

Table 3. Changes in components in Sugar C on storage at 55°C. (ppb on sugar)

Component	Baseline	1 Week	2 Weeks	6 Weeks
Color	26	28	33	38
Compounds that appeared to increase on storage				
Lactic acid	921	830	935	2431
PCA*	575	586	490	1146
Glycolic acid	112	75	99	196
Glyceric acid	30	46	48	105
Succinic acid	127	119	158	182
γ -Lactone-1**	18	29	22	68
γ -Lactone-2***	49	66	38	122
Compounds that did not appear to change significantly on storage				
Malic acid	83	66	86	92
Aconitic acid	18	36	15	20
Octanoic acid	8	25	8	11
Nonanoic acid	7	12	13	16
Palmitic acid	145	182	136	204
Linoleic acid	113	150	124	112
Vanillic acid	11	10	10	16
Oleic acid	54	66	45	39
Stearic acid	111	84	84	106
Compounds that appeared to decrease on storage				
Syringic acid	21	14	n.d.****	n.d.

* PCA = 2-pyrrolidone-5-carboxylic acid (pyroglutamic acid)

** γ -Lactone-1 = 2-methyl-2,3,4-trihydroxy-ribonic acid γ -lactone

*** γ -Lactone-2 = 3-deoxy-arabino-hexonic acid γ -lactone

**** n.d. = not detected

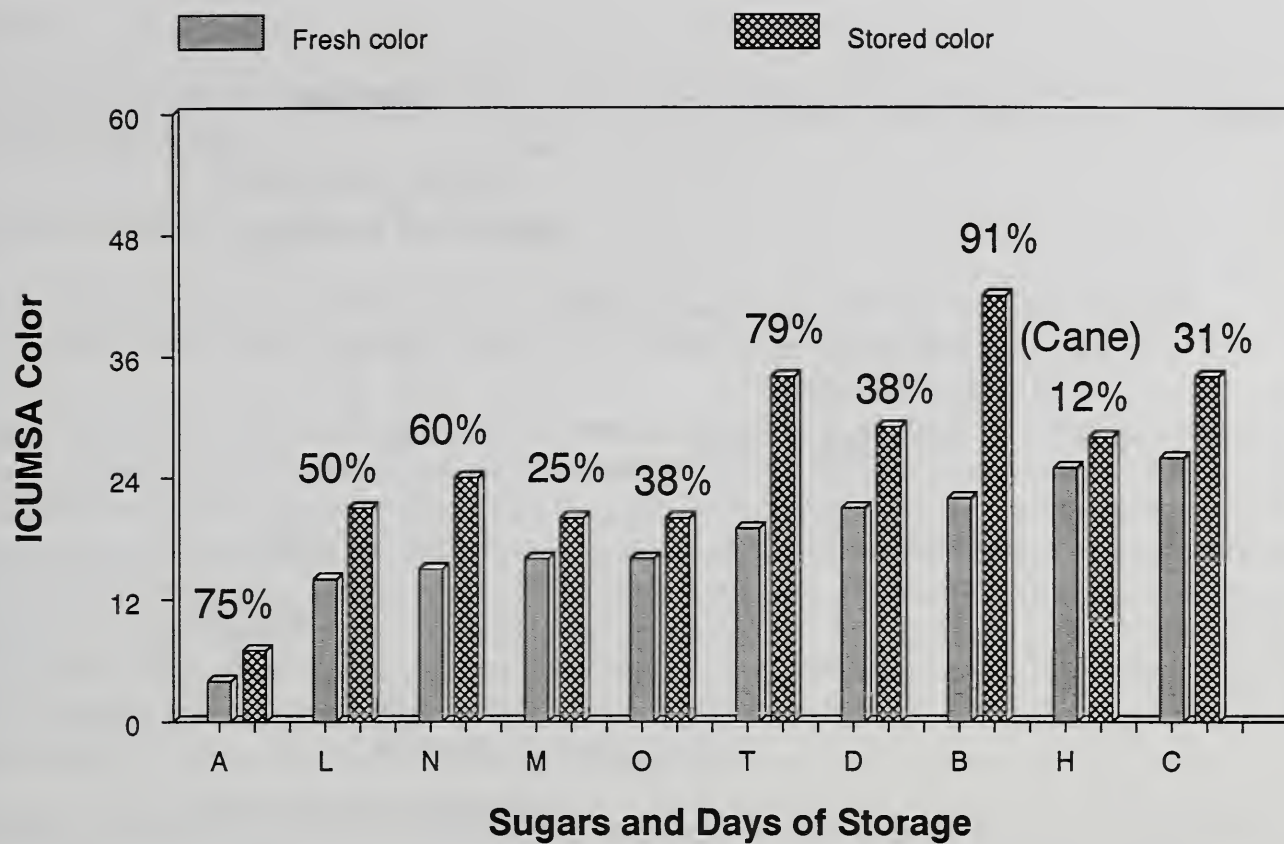


Figure 1. Changes that occurred in white sugars on storage at 55°C for 28 days.

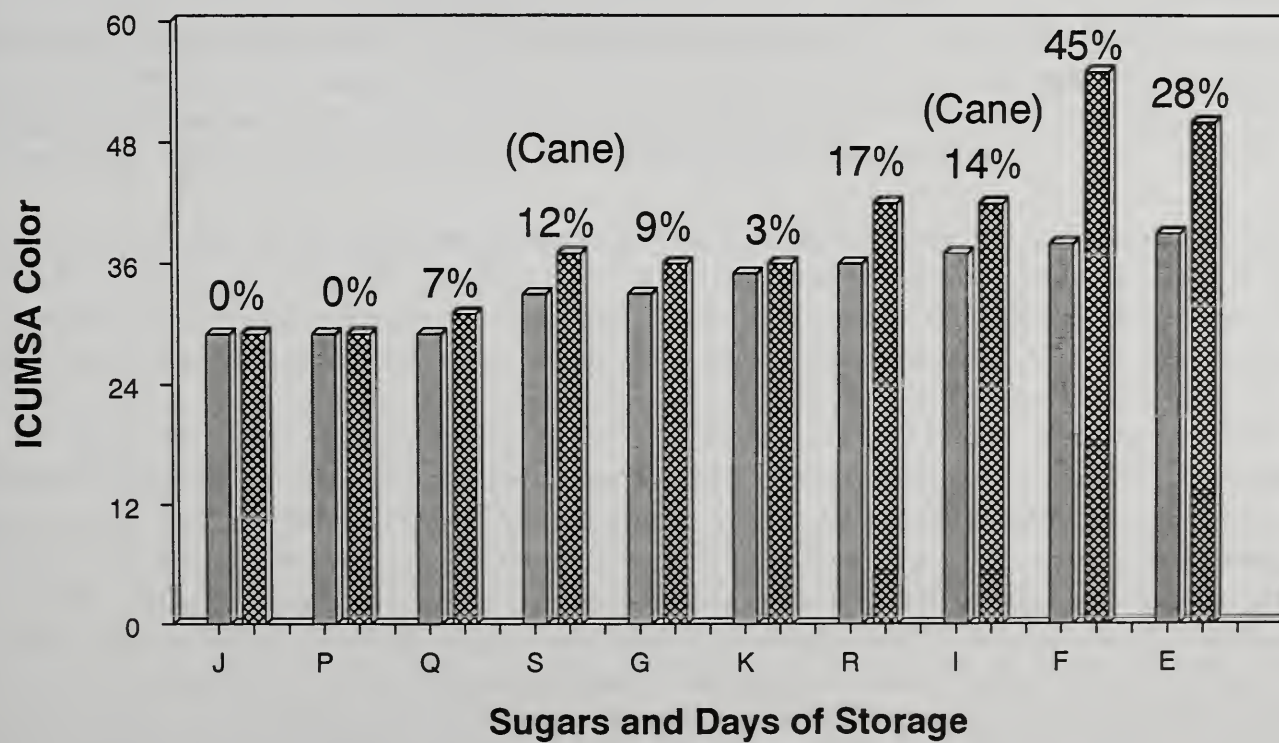


Figure 1 (cont'd). Changes that occurred in white sugars on storage at 55°C for 28 days.

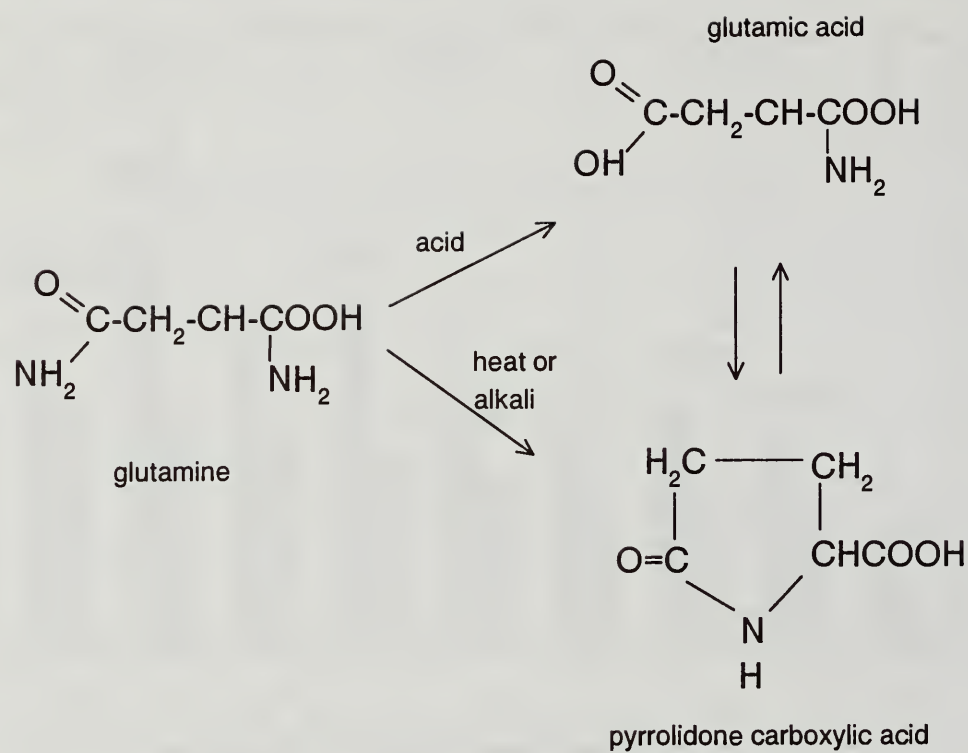


Figure 2. Formation of pyrrolidone carboxylic acid in process.

POSTER

AB INITIO QUANTUM MECHANICS AND MOLECULAR MECHANICS STUDIES OF A SUCROSE ANALOGAnne-Marie Kelterer¹ and Alfred D. French²¹Institut für Physikalische and Theoretische Chemie, Technical University of Graz, Austria²USDA-ARS-Southern Regional Research Center, New Orleans, Louisiana, USA

Many properties of sucrose are thought to depend on the shape of its molecules. Over the years, there have been many studies, both experimental and theoretical, of its 3-dimensional features. Perhaps the most characteristic aspect of any disaccharide is the relative orientation of the two monosaccharide rings, glucopyranose and fructofuranose, that are combined to make the non-reducing sucrose disaccharide (Figure 1). This relative orientation is expressed in terms of rotations about the bonds from the glucose and fructose rings to the linkage oxygen atoms. These two rotations, or torsion angles, can be calculated directly and precisely from some experiments such as x-ray and neutron diffraction crystallography. These torsion angles, named ϕ and ψ , can also be deduced from NMR experiments, and they can be predicted from different types of computerized molecular modeling studies.

In modeling studies, each of the torsion angles is changed over 360° in increments of, for example, 20° and the energy is calculated. For that size of step, there will be 18 different values each for ϕ and ψ , giving 18×18 or 324 different calculations of the energy. The energy at each grid point can be plotted to develop a three-dimensional surface that depicts how the energy changes with ϕ and ψ . The point on the surface having the lowest energy would be the most probable place to find an experimental structure, and experimentally determined points would be expected to have generally low energies. These energy surfaces, often shown as plots of iso-energy contours, provide a convenient way to understand various phenomena, including crystallization, and to make predictions of as yet unobserved behavior. They also are a good test of the modeling method when comparisons are made with experimental data.

The first such modeling work suggested that sucrose would be rigid (1) with its conformation being the one found in the crystal structure (2). However, it was realized that when sucrose crystallized in different environments that its shape changed. For example one could consider the shape in the sucrose part of a trisaccharide such as raffinose or erlose, or look at the shape in salt complexes. Also, there were numerous derivatives of sucrose. Even if the chemical changes were not likely to affect the linkage between the two rings, the shape often changed, presumably to fit into a different crystal lattice. Subsequent experimental NMR studies also showed that sucrose had substantial flexibility in solution (3). Therefore, new theoretical studies allowed the individual bonds and bond angles to relax in the modeling calculations (4,5) and some flexibility was indeed observed. Still, it was observed some years ago that, compared to many other disaccharides such as maltose and cellobiose, the available modeling methods did a poor job of predicting the shapes that would be found for sucrose moieties in other molecules or other environments (6). In particular the shape of the sucrose moiety in crystalline raffinose (7) had a very high energy (7.0 to 10.0 kcal or so) according to the available modeling information. It would be very improbable that a significant number of molecules in solution would have a conformation with such a high energy. If the energy

calculations were correct, the raffinose crystal would never have grown. Raffinose was not the only molecule with a crystal structure having a linkage conformation that corresponded to a high energy on the available energy surfaces. Many of the conformations observed in molecules relevant to the question of sucrose shape had high energies.

In the decade that followed the initial studies of sucrose with the Hard Sphere Exo-Anomeric effect (HSEA) program (5), computer technology advanced at an exponential pace. The "relaxed" studies, based on the MM3 (8), CHARMM (4) and PIMM88 (3) computer programs, would not have been practical in 1980, but by the early 1990's, such studies were fairly routine. All of these calculations were based on "molecular mechanics" formulations of the molecular energy. (The HSEA program was a simplified molecular mechanics program.) Molecular mechanics attempts to summarize what we know about the important details of molecular structure through equations for the energetic costs of, for example, stretching a bond from its ideal distance and bending bond angles from their ideal values. These equations, in their simplest form, resemble the equations for storing potential energy in a stretched or compressed spring, for example. That is why the technique is called "molecular mechanics". Other components of the molecular mechanics potential energy include deviations from ideal values for interatomic spacing when the atoms are not bonded to each other and the resistance to twisting about a given bond. This latter contribution is called the torsional potential. In the case of studies of sucrose, it became clear that these molecular mechanics summaries of what we think we know about the important aspects of molecular structure were inadequate. New theory would be needed before molecular mechanics could be used to create a satisfactory energy surface for sucrose.

One feature of carbohydrate structure that has received a lot of attention from theoreticians is the exo-anomeric effect (9). This "effect" is an unexpected pattern for the energy change when rotating about glycosidic bonds, such as the two bonds that link the glucose and fructose monomers of sucrose. Based on traditional concepts of structural chemistry, the four atoms that define a torsional rotation adopt the "anti" conformation, which places the two ending atoms as far apart as possible. An example is furnished by the simple butane molecule with its four sp^3 carbon atoms. When there are alternating oxygen and carbon atoms, O-C-O-C, such as found proceeding from the ring oxygen to the glycosidic carbon to the glycosidic oxygen to the aglycon carbon of a methyl glucoside, the situation changes. Then, the preferred torsion angle is "gauche", or only 60° , instead of the 180° , anti value. The MM3 program is designed to handle this situation. However, we have proposed that the problem with modeling sucrose arises because each of the monomers of sucrose contributes to a longer sequence, C-O-C-O-C-O-C. This longer sequence might have different torsional energies than found for the shorter sequences responsible for the ordinary exo-anomeric effect (10).

Although there have been many explanations for the anomeric and exo-anomeric effects over the years, the dominant cause is, according to most theoreticians, "hyperconjugation" which is a rearrangement of the lone-pair electrons on oxygen into anti-bonding orbitals when the orbitals on the various atoms are given different relative orientations. The most practical way to investigate changes in electron distribution, and their energetic and structural consequences, is to use quantum mechanics modeling. This method depends on approximate solution of the Schrödinger equation. The problem with quantum mechanics is that calculations that can be assumed to be reliable are very time-consuming and can require the biggest supercomputers for molecules smaller than glucose.

When we realized that the various molecular mechanics energy calculations for sucrose were inadequate (10), accessible computer technology was just sufficient to do quantum mechanics calculations for an analog of sucrose that had no hydroxyl or hydroxymethyl groups. A report of that work was presented in a poster at the 1994 S.P.R.I. Conference in Helsinki. Those calculations used HF/4-21G (d orbitals on oxygen atoms) theory, which was at the lowest level that we thought might give fairly reliable answers. Also, only a limited range of torsion angles was studied, so that the computer time would not be greater than we could afford. Still, these calculations were very encouraging, as all of the crystallographically observed structures corresponded to quantum mechanics energies of less than 3.0 kcal/mol, a reasonable result.

During the past ten months we have looked again at this problem. Only recently has it become feasible for us to perform such extensive calculations as we have now carried out. A Silicon Graphics Power Challenge computer with 12 processors was used, and we used HF theory and 6-31G basis functions with d orbitals on carbon and oxygen atoms. This is a widely accepted basis set, and energies from those calculations rival those from much more complete theory (11). Although we used the same analog (Figure 1) with tetrahydropyran and tetrahydrofuran rings (thp-thf), we were able to consider full 360° rotations about both bonds to the linkage oxygen atoms. The new energy map for the analog, which took about one cpu year, confirmed the 4-21G calculations in the limited region that was studied earlier. In most of the rest of the torsion-angle variation space, however, the results were surprisingly similar to the MM3 results for the analog. Subsequently, MP2 and B3LYP calculations were carried out. Those quantum mechanics calculations are more complete, incorporating corrections for electron correlation. These more-expensive calculations were carried out for only two conformations: at the minimum in energy near to the conformation from the crystal structure of sucrose, and for the saddle point near the conformation found for crystalline raffinose. They showed an even smaller difference in energy between the sucrose and raffinose conformations, but the dependence on level of theory was modest. The MP2 calculations, however, provided outstanding reproduction of the variable C-O bond lengths in the crystal structures (Figure 2). Therefore, we felt that the backbone of the sucrose molecule was represented well by the quantum mechanics calculations.

Although the *ab initio* energy surface was vastly superior in its accounting for the observed crystal structure conformations, compared to the MM3 surfaces for either the analog or the full sucrose molecule, it was still not an attractive match. The main problem, from the standpoint of the results, is that the global minimum on the new HF/6-31G* surface is not populated by any observed crystal structures. Another problem is more philosophical. The thp-thf analog of sucrose is not sucrose, and a valid map should include all of the atoms actually in the sucrose molecule.

Although we are interested in contributing to the understanding of this problem, an important goal of the work is the determination of the correct energy surface for sucrose. To quickly determine that surface, given the *ab initio* surface for the thp-thf analog, we used an admittedly unusual procedure. We subtracted the MM3 energies for the analog from the MM3 energies for sucrose and then added the *ab initio* energies for the analog to produce the energy surface shown in Figure 3. This procedure essentially uses the *ab initio* thp-thf calculations to provide the information for the overlapping exo-anomeric effect, and the full MM3 calculation provides the energies of the interactions of the hydroxyl and hydroxymethyl groups.

We consider the resulting MM3-ab initio map to be the most correct yet obtained for sucrose, primarily based on its correspondence with structures obtained by diffraction crystallography. Twelve of the experimental conformations are within the 1.0 kcal/mol contour, six are between 1.0 and 2.0 kcal/mol and three are between the 2.0 and 3.0 kcal/mol. The raffinose conformation corresponds to 4.87 kcal/mol on the corrected map but about 7.64 kcal on the pure MM3 map. The other outlier, at about 4.10 kcal, is trichlorogalactosucrose (12). Its energy was increased by about 0.8 kcal by the above energy correction procedure. Extensive study showed that raffinose had no internal features that would cause a high-energy shape to be preferred (10), nor was crystal packing likely to be responsible for a high energy conformation.

On our uncorrected MM3 map for sucrose, the average energy for 23 crystal structure conformations is 2.43 kcal/mol. On the map corrected by the ab initio calculations the average corresponding energy is 1.32 kcal/mol. The ideal value, if the crystal structure captured a Boltzmann distribution of conformations having two degrees of freedom at 300°C with no strain, would be 1.19 kcal. Although the very good agreement between the Boltzmann energy and the average conformational energy does not prove that the corrected surface is the "true" surface, it suggests that any further improvement will not come from attempts to improve the correspondence with the currently available set of crystalline conformations.

We envision that the ultimate energy surface for sucrose will have an improved fit for the raffinose conformation and perhaps will also accommodate the trichlorogalactosucrose structure at a lower energy value. The sucrose energy surface is especially difficult compared to other disaccharides, not only because of the overlapping exo-anomeric effect but also because of the flexible five-membered fructofuranose ring. As the furanose ring changes shape, part of the C-O-C-O-C-O-C sequence changes its shape, with consequent variations in the energy changes for the remainder of the torsion angles. We also look forward to learning the reasons for the unusual behavior at the sucrose linkage.

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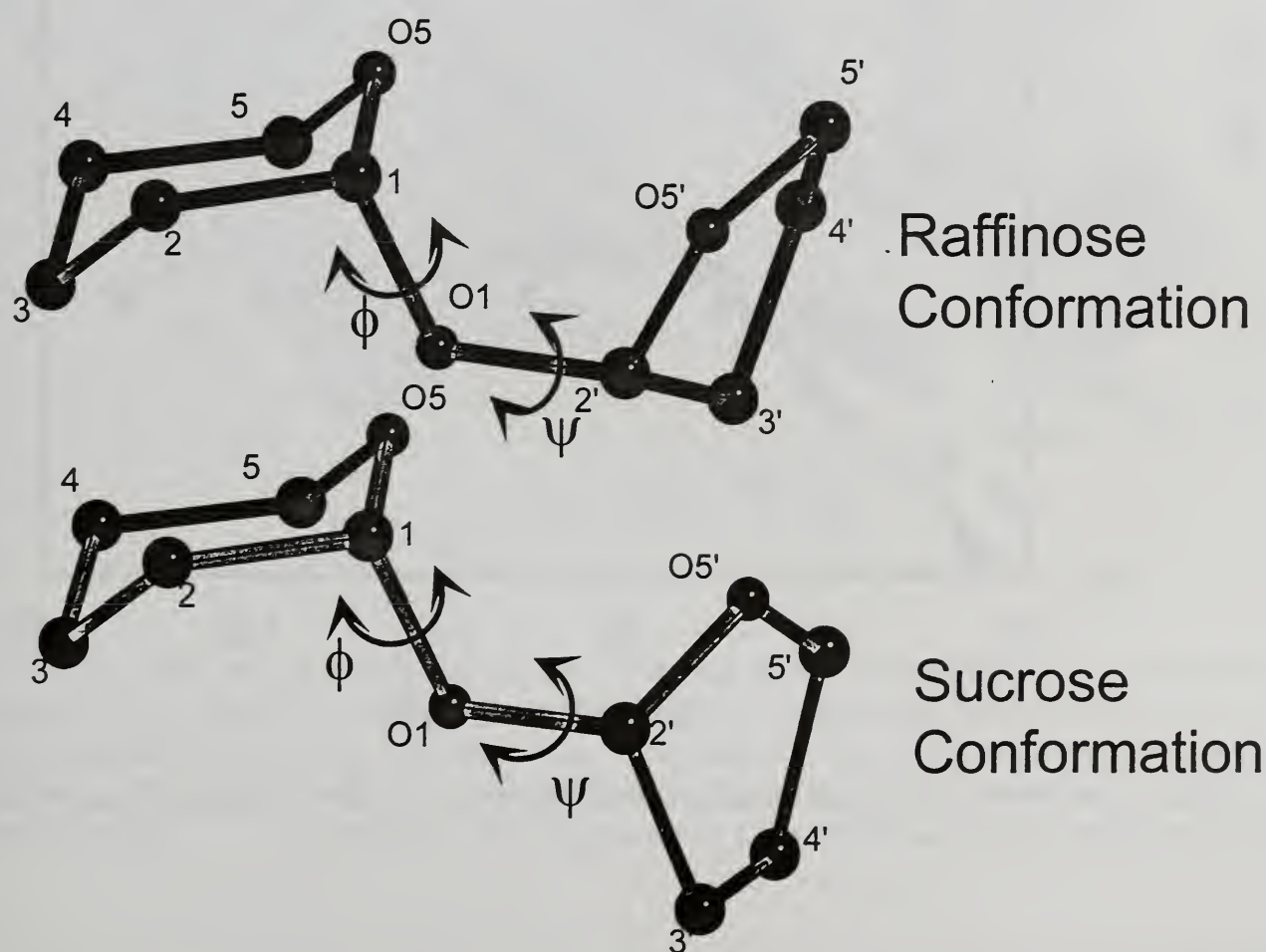


Figure 1. The tetrahydropyran-tetrahydrofuran (thp-thf) analog of sucrose in both the sucrose and raffinose conformations. The carbon atoms are numbered, and the oxygen atoms also show the letter O. Hydrogen atoms are not shown. The torsion angles ϕ and ψ indicate the amount of rotation about the two glycosidic bonds, C1-O1, and C2'-O1. They are defined for the sucrose linkage by their respective ring oxygen atoms: $\phi = \text{O5-C1-O1-C2'}$, and $\psi = \text{O5'-C2'-O1-C1}$.

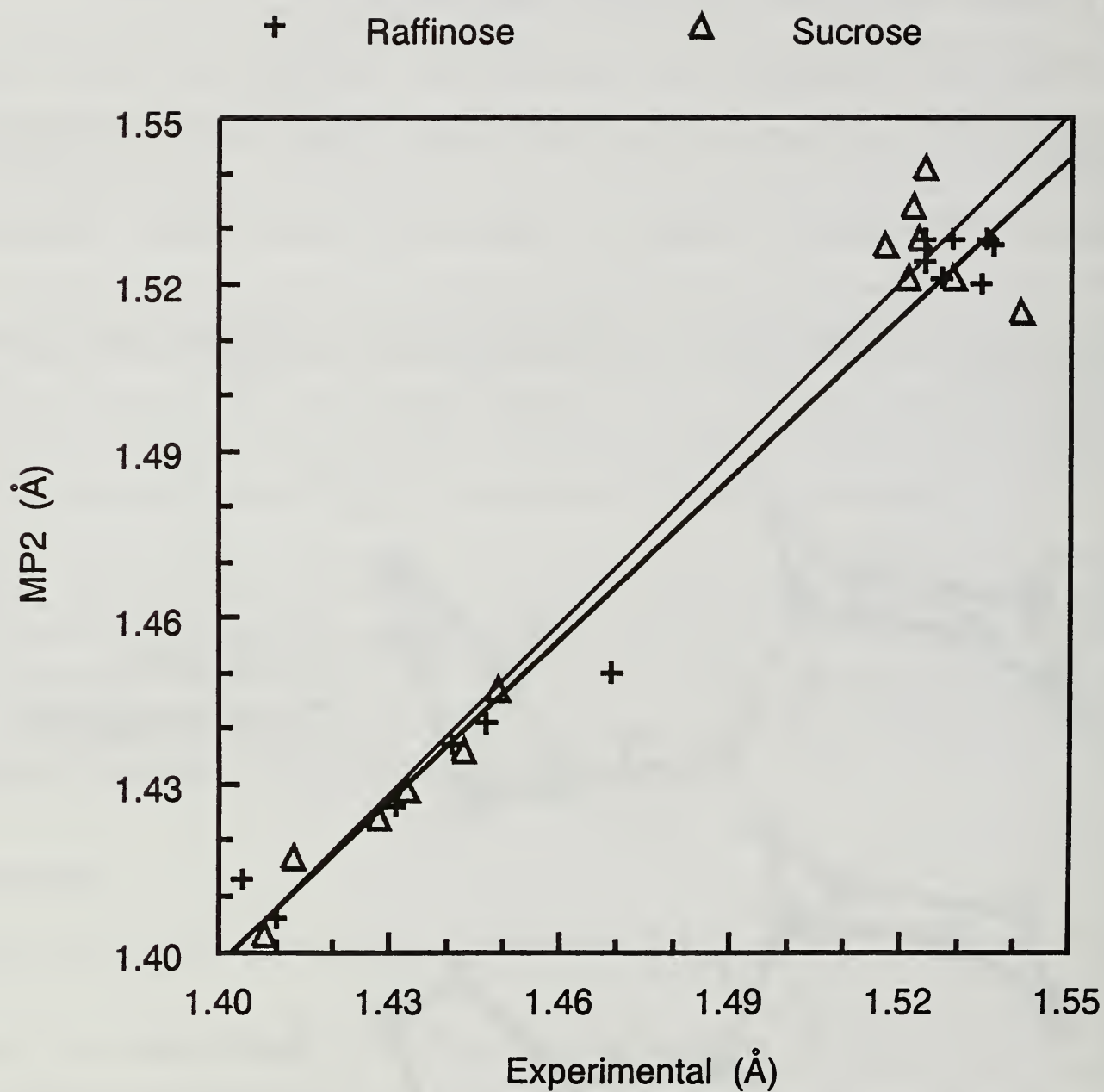


Figure 2. The comparison of the bond lengths derived from the crystal structures of sucrose (2) and raffinose (7) and those calculated by MP2/6-31G* theory for the thp-thf analog.

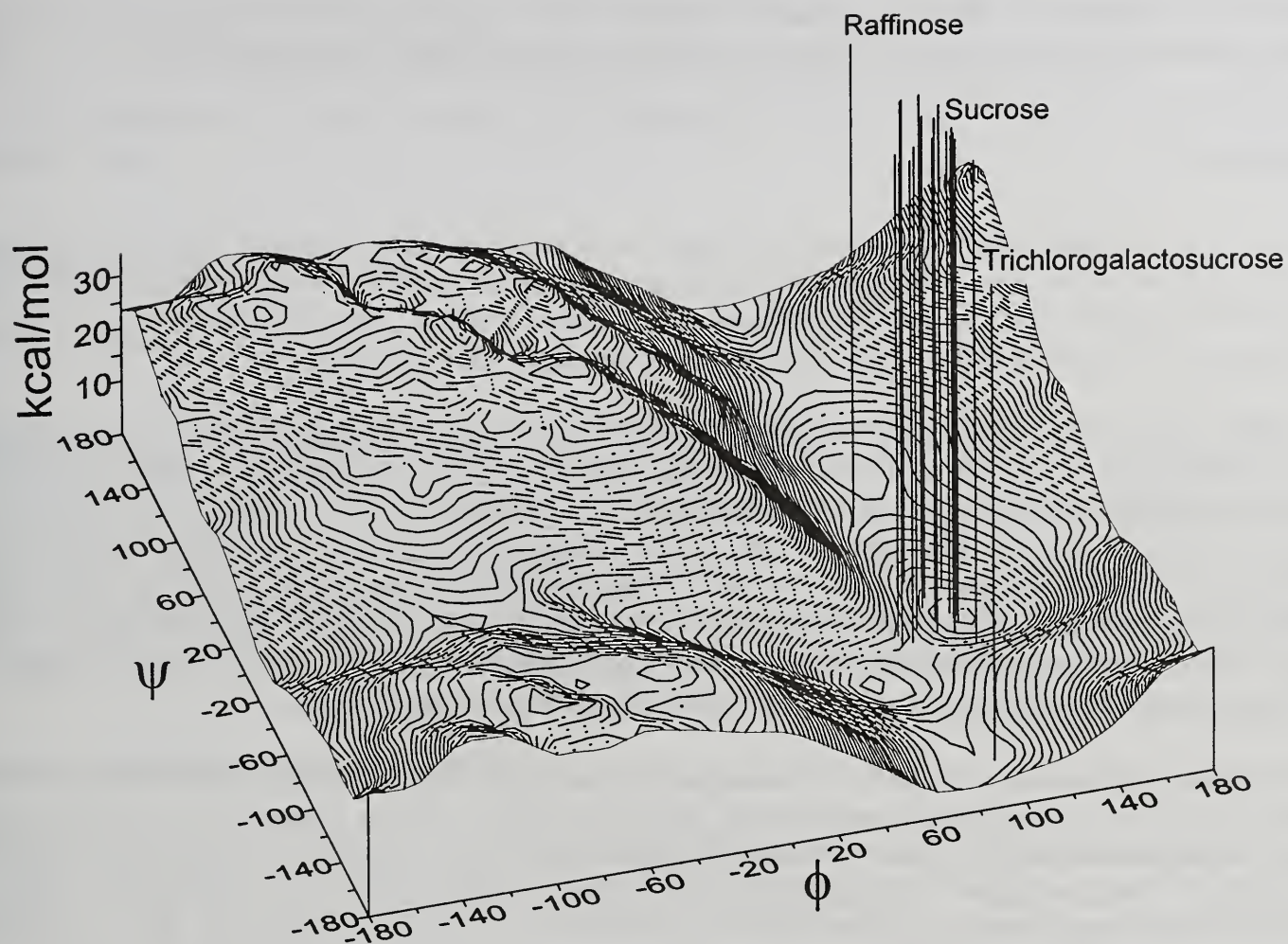


Figure 3. The MM3 energy surface for sucrose, corrected by ab initio calculations. The vertical lines correspond to the conformations observed in various crystal structures that include the sucrose moiety, or at least a reasonable analog of it. The line closest to the reader is for trichlorogalactosucrose, and the line most distant is for raffinose, with the line for sucrose being in between. Contour lines are drawn at 0.5 kcal/mol intervals.

POSTER

APPROACHES FOR THE REDUCTION OF WHITE SUGAR ODOR

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ABSTRACT

Several ways to reduce or prevent white sugar odor were explored. One approach was to use alternate sources of factory water for centrifugal wash. Different evaporator condensates were used in the final wash step and the washed sugar evaluated via sensory panel. It was found that switching to alternative factory condensates as sources of wash water has little, if any, apparent effect on sugar odor.

The effect of wash water on sugar odor was tested another way. Sugar was crystallized from standard liquor in a pilot-scale pan. Half of the sugar crop was washed in the centrifugal with purified, research-grade water ($\sim 16\text{--}18\text{ mega}\Omega$); the remaining sugar was washed with factory condensate water. Water temperatures were $\sim 100^\circ\text{C}$. The washed sugar was recovered, dried, then evaluated for odor by sensory panel. In most cases ($\sim 67\%$), sugar washed with pure water had lower odor than sugar washed with condensate. However, sugar washed with pure water still had a detectable odor, although it was of a noticeably different character than sugar washed with condensate. Odor in the former sugar is probably due to odorants which are trapped in the sugar during crystallization, and which eventually diffuse out of the crystals.

Aeration was also tested as a means of removing odor from sugar. Sugar obtained from the cooler in the factory was placed in a cylindrical conditioning bin and aerated at ambient temperature with purified air. Sugar samples were withdrawn after 0, 24 and 48 hours of aeration and evaluated for odor via sensory panel. In a total of 17 trials, aeration was found to produce a positive effect on odor in 11 (65%) of the samples tested. In most cases, a significant odor reduction was observed after 24 hours of aeration. The data suggest that odor reduction of sugar in a factory can be accomplished through the use of a conditioning silo.

INTRODUCTION

Beet sugar has long been known to have a characteristic odor. Conditions that contribute to this odor include bacterial infections, poor beet quality, unfavorable beet storage conditions, incomplete beet washing, odorants in factory air and process streams, etc. (2, 3, 7).

Beet sugar odor has several descriptors such as grassy, "green," barn-like, rancid, sour, earthy and musty (2). In recent years, several laboratories have studied the causes of sugar odor and have identified some of the substances responsible for this problem. For example, organic acids and aldehydes were identified by Godshall (6) and Godshall et al. (7). These can impart sour and grassy odors, respectively, to sugar. Geosmin, an earthy/musty-smelling microbial metabolite, was identified in beet sugar by Marsili et al. (10). Pyrazines have also been found in beet sugar and beet factory process streams (10, 12). Some pyrazines

have nutty or roasted aromas, while others have earthy odors. Other (as yet unidentified) volatiles may also contribute to sugar odor. The odor character of a specific sugar probably depends on the types and amounts of different odorants present in the sample.

American Crystal has studied beet sugar odor for several years (3). Even though the odor does not negatively impact final products, there is a perception that it does. For this reason, we have recently focused on ways to reduce or eliminate odor from white sugar. These included aeration of sugar in a conditioning bin and using different sources of water for the centrifugal washing step. A report of our findings is presented below:

MATERIALS AND METHODS

Water Analyses

Centrifugal wash water (CWW) and evaporator condensate water samples were collected on site at one of American Crystal's sugar factories (hereafter referred to as "Factory A"). As sample temperatures were @~100°C, the water was first chilled by passage through a cooling apparatus consisting of a stainless steel coil contained within a water jacket through which cold water was circulated. This was done to prevent "flashing" of volatiles from the sample during the collection process. Water entered and exited the cooling device via stainless steel hoses and was collected in acid-washed glass bottles. Water leaving the apparatus was @~room temperature. Samples were stored in a cold room until analyzed.

Geosmin and Methyl-Isoborneol (MIB)

Water samples collected as above were shipped under refrigeration to the Philadelphia Suburban Water Company in Bryn Mawr, PA. There they were analyzed for geosmin and MIB by a procedure known as closed-loop stripping wherein volatiles are purged from water samples with an inert gas, then adsorbed on a carbon trap. This is extracted with CS₂ and the extract analyzed by GC. Identities of unknowns are confirmed by mass spectrometry.

Volatile Fatty Acids (VFA's)

VFA levels in water samples were quantitated by ion chromatography (IC). Samples were filtered through 0.45μ syringe filters into IC vials, then analyzed at ambient temperature on a Dionex DX-300 ion chromatograph with a 4 mm Dionex Ionpac AS-11 column fitted with an AG-11 guard column. Separation was by gradient elution (@1 ml/min) with distilled water, 5 mM- and 10 mM NaOH as mobile phases. Instrument calibration was with a mixture of VFA standards. Detection/quantitation of VFA's was via conductivity (sensitivity @ ~0.5 ppm).

Aeration of Sugar

Aeration of sugar was accomplished by means of a conditioning bin (Figure 4). A cylindrical chamber (14-in. diameter) with a conical lower section was fabricated from stainless steel by Standard Industries, Inc., Fargo, ND. This was mounted on a heavy-gauge steel laboratory cart and fitted with a Dayton Model 2C820 blower powered by a Dayton Model 3N855 ½-hp 3450 rpm motor. For air purification purposes, a charcoal filter was fitted over the air intake of the blower. Air from the blower was directed to the conical section of the conditioning chamber through a 4-in. diameter PVC pipe. A perforated high-density polyethylene disc covered by a nylon screen (40 μ mesh) was positioned between the conical and cylindrical bin sections to serve as a rigid support for the sugar. The upper end of the bin was covered with a removable screen fabricated from furnace filters and was used to exclude foreign matter from the bin during operation. Air flow rates were measured with an Alnor CompuFlow Model 8565 Thermo Anemometer. The bin was assembled by the Research Center's Facility Services Staff.

Conditioning tests were performed by filling the bin to a depth of 7-8 inches with ~30-39 lb of sugar freshly obtained from the cooler at Factory A, then blowing ambient air through the sugar bed at rates of 10 - 27 ft³/min. Sugar samples were taken after 0, 24 and 48 hours of aeration and stored at -20°C in sealed 8-qt stainless steel kettles until evaluated by sensory panel.

Wash Water Quality and Sugar Odor

Effect of Alternating Condensate used for Centrifugal Wash Water (CWW)

A changeover to the condensate of choice was made at Factory A 24 hours in advance. After this time, sugar samples were collected from the cooler in clean plastic bags every hour for 8 hours. The sugar was later combined and mixed in a large stainless steel pot to yield a composite sample representative of sugar produced over an 8-hr shift. On the next day, condensate from a different evaporator was brought on line for use as CWW, and the entire sampling and compositing process was repeated. Sugar samples obtained in this way were then evaluated by sensory panel.

Use of Purified Water for Centrifugal Wash

This was tested by crystallizing sugar from standard liquor in a pilot scale pan. Two "strikes" (each representing ~50% of the sugar) were obtained from the pan. In the first strike, the sugar was washed in the centrifugal with ~450-520 ml of purified, research-grade water (15.9-17.8 mega- Ω); the remaining sugar was washed in an identical manner using sugar factory condensate. Washing was done using a pressurized plastic garden sprayer equipped with an adjustable spray nozzle. Wash water volumes were monitored via a Mettler PM34-K Delta Range balance. In most cases, wash water temperatures were ~100°C. The washed sugar was recovered, dried to <0.04% moisture in an Ultra-Air Baker's Aid Mini-Oven for 20 min @ 75°C, then evaluated by sensory panel.

Sensory Evaluation

Sugar samples (~65 g) were sealed into 4-oz Qorpak jars with teflon-lined lids and kept at room temperature for 24 hours to allow sugar odorants to fill the headspace in the jar. The sugar was then evaluated by a trained, 18 - 22 member sensory panel. Sugar odor was rated for intensity and degree of unpleasantness on either a 10- or 20-point scale. Statistical analyses were performed on a computer using Statgraphics Plus.

RESULTS

PREVENTION OF SUGAR ODOR

Identify and Use the Best Available Condensate for CWW

Centrifugal wash water (CWW) contains numerous organic compounds, some of which have the potential to impart odor to sugar during the wash cycle. These include organic acids, geosmin and other substances (3, 6, 7, 10, 12). If these are removed from the wash water prior to use, the sensory quality of sugar might be improved. If this is not possible, then perhaps using factory process water with the least amount of odorants for centrifugal wash might reduce sugar odor. These approaches have been suggested by previous investigators (2). For this reason, attempts were made to identify the best available condensate for use as wash water.

Water samples were collected from the evaporators at Factory A on five separate occasions during the fall of 1996 and analyzed for odorants such as methylisoborneol (MIB), geosmin and volatile fatty acids (VFA's). The latter are responsible for rancid, sour odors, while geosmin and MIB are earthy, musty odorants.

None of the samples were found to contain MIB, and only low levels of VFA's were detected. However, varying amounts of geosmin were found in all of the waters tested (Figure 1 & Table 1). In particular condensate 2A was found to contain geosmin in amounts well in excess of threshold (10-20 parts/trillion); above-threshold levels were also found in several of the other samples tested. The data show that geosmin concentrations can vary at different times during the campaign and with the water source.

Acetic acid was the only VFA consistently present in the samples tested; however, the levels were well below the reported odor threshold for this substance (7). Iso-butyric, valeric and iso-valeric acids were absent. Very low concentrations of propionic, formic, butyric and caproic acids were occasionally found in a few samples (data not shown). It is believed that VFA's are mainly responsible for beet sugar odor (7); however, geosmin may also contribute (10).

The differences in geosmin levels suggested that certain condensates might be better than others for use during the centrifugal wash step. For this reason, the effect of using different condensates as CWW on sugar odor was explored. This involved collecting and evaluating sugar washed by different water sources (Methods). The evaluation process necessitated the formation, training and maintenance of a sensory panel

composed of dedicated volunteers from within the Research Center. Data from the panel were analyzed by a trained statistician.

Data (not shown) from initial trials suggested that condensate #3 was better than condensate #1. This phenomenon was more thoroughly tested. With the cooperation of Factory A personnel, a changeover to the condensate #1 was made 24 hours in advance of sampling. Then, samples of sugar washed with condensate #1 were collected, brought to Research, composited and stored in stainless steel containers (Methods). On the following day condensate #3 was used as CWW, and the entire sampling and compositing process was repeated. The sugar samples were later tested by sensory panel. In all, four such trials were conducted.

The results (Table 2) show that in three of the four trials, there was no significant difference in sensory quality of the sugar regardless of the wash water source. Although there may be variations in chemical make-up between the condensates, these are apparently not large enough to significantly change the odor levels of sugars washed by water from these sources. As such, there appears to be no significant benefit in switching wash water sources within the factory.

Sugar Odor as a Function of Wash Water Quality

The effect of wash water source on sugar odor was examined in another way. Assuming that odorants in wash water affect sensory quality, then sugar washed with pure water should have no odor, or less odor than sugar washed with condensate. Conversely, if washing sugar with pure water does not eliminate odor from the crystals, then there is no justification for switching sources of wash water or for installing a treatment system in the factory to purify the latter.

This hypothesis was tested by crystallizing sugar from Factory A standard liquor in a pilot scale pan at Research (Figure 2). Two "strikes" (each with ~50% of the sugar) were obtained from the pan. In the first strike, the sugar was washed with pure water; the remaining sugar was washed in an identical manner with Factory A condensate #1. The washed sugar was recovered, dried, then evaluated by sensory panel. The results are shown in Table 3.

In the first two trials, sugar washed with pure water had less odor than that washed with condensate, although the differences were not significant. However, in trials 3-6, sugar washed with factory condensate had statistically higher odorant levels than sugar washed with pure water. The data indicate that wash water quality does influence sugar odor. It is important to note, however, that sugar washed with pure water was not devoid of odor. Rather, it had a detectable odor that was of a noticeably different character than that of sugar washed with condensate. Whereas the latter sugar had a "boiled corn" odor typical of wash water and condensates, sugar washed with pure water had an odor reminiscent of wet, painted wood/cardboard. Moreover, in at least two sensory trials, sugar washed with pure vs. factory water could be identified based on odor alone.

REMOVAL OF ODOR FROM SUGAR

Conditioning of Sugar by Aeration

It was decided to determine if conditioning sugar with clean air could reduce sugar odor. This strategy has been suggested by other investigators (2). The premise for this approach is that if odorants are localized within or on the surfaces of sugar crystals, it should be possible to remove these by aeration with clean air. Evidence to support this hypothesis was obtained from sugar samples that were repeatedly subjected to sensory analysis over a period of several weeks. The results of these tests (Figure 3) show that the odor intensity decreases over time due to "flashing" of odorants to the atmosphere.

Sugar conditioning was accomplished by means of a cylindrical, stainless steel conditioning bin fitted with a blower (Figure 4). The bin was installed in a restricted area of the Research Center, away from any sources of airborne odorants (e.g., chemicals, cigarette smoke, etc.). Conditioning trials were performed as described (Methods and Figure 5). After aeration, sugar was withdrawn from the bin and stored at -20°C until evaluated by sensory panel. A sample of the original unaerated sugar (stored as above) served as a control.

Table 4 shows that, in a total of 17 trials, aeration was effective in 11 (~65%) of the samples tested. Note that in the latter trials, aeration times were reduced from 48 to 24 hours. At the completion of trials at Research, the bin was relocated to Factory A and set up in the Cooler/Granulator Room for additional testing to determine if conditioning would be efficacious in a factory setting. Twelve trials were conducted as described above. Table 5 shows that significant reductions in odor were obtained in 8 of the 12 trials (67%). The data agree with the results of conditioning trials performed at Research and show that aeration can reduce sugar odor even in a factory setting, where air quality is usually lower.

CONCLUSIONS

From the foregoing data, it was possible to draw several conclusions:

Switching to alternative condensates as sources of wash water in the factory has little, if any, apparent effect on sugar odor intensity.

Sugar washed with pure water has less odor than sugar washed with factory condensate. \therefore Wash water quality does impact the sensory properties of sugar.

Sugar washed with purified water can still have an odor. This is probably due to odorants present in syrup. These are occluded within the sugar during crystallization and eventually diffuse out of the crystals.

Since sugar washed with pure water has an odor different from condensate-washed sugar, it would appear that odorants in CWW are different from those localized within the crystals. This is supported by the findings of others (2).

Sugar odor intensity decreases over time as a result of “flashing” of odorant molecules from the crystals to the atmosphere. Conditioning with clean air accelerates this process.

OTHER PREVENTATIVE MEASURES

Air Treatment Systems

At times, the quality of the air in a sugar factory is poor. Sugar is known for its ability to adsorb airborne odorants. Thus, a contributing factor to sugar odor could be adsorption of odorants onto the surfaces of sugar crystals. Removal of odorants from the air, especially in the Granulator/Cooler Room and packaging areas, might minimize this possibility. Industrial-scale air treatment systems are commercially available. Two of these are described below:

Regenerative Thermal Oxidizer (Durr Industries, Plymouth, MI)

This works by drawing airborne odorants into a combustion chamber for incineration and releasing odor-free air to the surroundings. The device recovers most of the heat of combustion, reducing energy consumption by >90%.

Re-Gensorb System (M & W Industries, Rural Hall, NC)

In this device, odorants are passed through a bed of zeolite where they are trapped; air exiting the system is odor-free. At intervals, odorants are desorbed from the zeolite and burned, thereby regenerating the adsorbent.

These devices are used in many manufacturing facilities in the US for the removal of hazardous solvent vapors, paint fumes, dust particles, etc. Each of these devices costs ~\$1 million for a factory-scale unit. Both manufacturers have pilot-scale units available for on-site trial purposes. Neither of the devices has been evaluated by American Crystal Sugar.

Treatment of Water used for Centrifugal Wash

Centrifugal wash water contains many organic compounds which can affect sugar quality. If these are removed from the water prior to use, the sensory quality of sugar might improve. Treatment would involve passing the water through adsorbents such as charcoal, ion exchange and/or adsorbent resins, etc. Since CWW is used at ~105°C, and since adsorbent efficiency is inversely proportional to temperature, treatment requires that the water first be cooled to an acceptable temperature, then reheated for use in the centrifugals after adsorbent treatment. In the interest of heat economy, heat exchangers would be required. A system for this purpose is available from Alfa-Laval Thermal for ~\$9000 and would provide 20,000 gal of water

per day. Pilot-scale tests would be necessary to identify resins of suitable efficiency for odorant adsorption. Resin costs could range from \$21,000-\$180,000.

DISCUSSION

During the course of this study, it was demonstrated that using different condensates as sources of CWW has no significant effect on the intensity of sugar odor. However, washing sugar with purified water does improve sugar quality. Similarly, conditioning sugar by aeration clearly reduces sugar odor intensity

Since wash water quality does affect sugar odor intensity, use of the purest water available for centrifugal wash in the factory should have a beneficial effect. However, as described above, this cannot be achieved by switching condensates; rather, a cleaner wash water is needed. This requirement can be met by increasing a factory's daily usage of clean city water (if available) or by use of a treatment system to purify CWW. It is important to note, however, that in our experience, use of purified water for centrifugal wash did not entirely eliminate sugar odor. The sugar so produced had an odor, although it was of lower intensity and of different character than that of sugar washed with condensate. Use of purified water for this purpose would probably improve sugar quality. However, more tests are needed to determine if this treatment by itself produces a significant (vs. marginal) improvement; otherwise, the cost of implementation may not be justified. A combined approach using purified CWW followed by conditioning with clean air (see below) is another interesting option that could be explored.

The observation that sugar made at Research and washed with pure water still has an odor is important for another reason, as it suggests that the contribution to sugar odor by factory air is minimal. Although there are devices by which factory air can be purified, the data presented here suggest that this approach may produce only marginal results.

The data obtained from this investigation indicate that the best option for reducing sugar odor would be realized by the construction and implementation of an industrial-scale sugar conditioning silo. Additional pilot-scale trials are necessary to determine minimum aeration rates and times necessary for maximum odor reduction. Systems for humidity control and air filtration need to be tested. A conditioning silo would be a costly investment; however, the data presented here indicate a high probability of success. A secondary benefit from conditioning sugar would be a reduction of hard/lumpy sugar complaints from customers (11, 13). This would help to make the Return on Investment more attractive.

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Table 1. Geosmin and organic acid levels in Factory "A" water samples.

<u>Collection date</u>	<u>Water source</u>	<u>ANALYTE</u>	
		<u>Acetic acid (PPM)</u>	<u>Geosmin (PPT)</u>
10/24/96	Wash water	0.6	1.5
	Condensate #1	0.6	0.5
	Condensate #2A	1.3	262
	Condensate #2B	1.3	31.0
	Condensate #3	2.7	10.7
11/19/96	Wash water	1.9	1.2
	Condensate #1	0.5	ND
	Condensate #2A	2.2	123
	Condensate #2B	2.0	18.6
	Condensate #3	4.0	6.6
12/3/96	Wash water	1.2	0.2
	Condensate #1	ND	0.4
	Condensate #2A	4.6	31.2
	Condensate #2B	4.6	14.2
	Condensate #3	9.0	12.1
12/10/96	Wash water	0.7	0.2
	Condensate #1	0.8	0.4
	Condensate #2A	1.8	34.9
	Condensate #2B	1.7	7.2
	Condensate #3	2.2	7.5
12/19/96	Wash water	NT	2.1
	Condensate #1	NT	NT
	Condensate #2A	NT	17.5
	Condensate #2B	NT	28.3
	Condensate #3	NT	3.0

Methylisoborneol, i-Butyric, i-Valeric and Valeric acids: not detected

PPM = parts per million

PPT = parts per trillion

ND = not detected

NT = not tested

Geosmin odor threshold = ~ 10-20 ppt

Water samples were collected as described (Methods). Geosmin was quantitated by the Philadelphia Suburban Water Company, Bryn Mawr, PA, using closed-loop stripping followed by GC-MS. VFA's were analyzed by IC.

Table 2. Effect of wash water source on sugar odor: summary of statistical data.

Trial #	Condensate used	Sensory score*	Significant difference
1	#1	5.67	Yes
	#3	3.67	
2	#1	3.87	No
	#3	4.28	
3	#1	3.14	No
	#3	3.74	
4	#1	3.90	No
	#3	3.78	

*Sensory score: 0 = no odor; 10 = high odor

Sugar samples were collected at Factory A after having been washed with either Condensate #1 or Condensate #3 (Methods). Odor intensity was scored by a sensory panel.

Table 3. Effect of water quality on sugar odor: pure water vs. Factory "A" Condensate #1.

Trial #	Wash water used	Sugar* sensory score**	Significant difference
1	Pure water	3.85	No
	Condensate	4.6	
2	Pure water	4.78	No
	Condensate	5.56	
3	Pure water	3.97	Yes
	Condensate	5.63	
4	Pure water	4.44	Yes
	Condensate	5.91	
5	Pure water	4.03	Yes
	Condensate	5.34	
6	Pure water	3.16	Yes
	Condensate	4.5	

*Sugar crystallized from Factory "A" standard liquor

**Sensory score: 0 = no odor; 10 = high odor

Sugar was crystallized in a pilot-scale pan from Factory A standard liquor (Methods). Half of the sugar was washed with pure water; the remainder was washed with Factory A condensate. When dry, the sugar was scored for odor by a sensory panel.

Table 4. Results of sugar bin conditioning trials at ACS' Research Center.

<u>Trial #</u>	<u>Conditioning time (hrs)</u>	<u>Sensory score*</u>	<u>Significant difference</u>
1	0	6.67	Yes
	24	4.25	
	48	4.11	
2	0	6.69	No
	24	5.72	
	48	5.78	
3	0	7.17	Yes
	24	5.61	
	48	6.19	
4	0	5.36	Yes
	24	4.67	
	48	3.5	
5	0	6.39	Yes
	24	4.67	
	48	4.22	
6	0	4.78	No
	24	4.28	
	48	4.22	
7	0	6.33	Yes
	24	5.31	
	48	5	
8	0	5.67	Yes
	24	4.72	
	48	4.22	
9	0	6.08	Yes
	24	3.11	
10	0	4.68	No
	24	4.25	
11	24	6.18	Yes
		3.93	
12	0	5.3	No
	24	4.45	

Table 4. (continued)

<u>Trial #</u>	<u>Conditioning time (hrs)</u>	<u>Sensory score*</u>	<u>Significant difference</u>
13	24	5.18 2.3	Yes
14	0 24	5.25 3.75	Yes
15	0 24	4.48 3.18	Yes
16	0 24	4.98 4.48	No
17	0 24	5.88 5.34	No

*Sensory score: 0 = no odor; 10 = high odor

Conditioning tests were performed by filling the bin to a depth of 7-8 inches with 30-39 lbs of sugar from the cooler at Factory A, then blowing ambient air through the sugar at rates of 10-27 ft³/min. Sugar samples taken after 0-, 24- and 48-hrs of aeration were stored -20°C in sealed 8-qt stainless steel kettles until evaluated by sensory panel.

Table 5. Results of sugar conditioning trials at Factory A.

Trial #	Aeration time (hrs)	Odor intensity score*	Unpleasant odor score	Significant difference
1	0	5.5a**	n/a	Yes (P = 0)
	24	4.8a	n/a	
	48	2.7b	n/a	
2	0	5.1a	n/a	Yes (P = 0.0057)
	24	4.5a	n/a	
	48	2.9b	n/a	
3	0	5.9a	6.3a	Yes (P = 0.0007)
	24	3.8b	3.7b	
	48	4.3b	4.1b	
4	0	4.1	4	No (P = 0.37)
	24	4.7	4.7	
	48	4.8	4.8	
5	0	6.9a	6.8a	Yes (P = 0.0084)
	24	5.1b	4.6b	
	48	4.9b	4.5b	
6	0	5	5.5	No (P = 0.37)
	24	4.4	4.4	
	48	5.2	5.2	
7	0	4.5	4.3	No (P = 0.72)
	24	4	3.5	
	48	4.1	3.8	
8	0	4.4a	4.5a	Yes (P = 0.0026)
	24	3.9a	3.6ab	
	48	2.9b	2.7b	
9	0	4.9a	5.2a	No
	24	4.1a	4.6a	
	48	4.9a	5.3a	
10	0	5.7a	5.8a	Yes (P = 0.0088)
	24	3.7b	3.8b	
	48	4.2b	4.3b	

Table 5. (continued)

Trial #	Aeration time (hrs)	Odor intensity score*	Unpleasant odor score	Significant difference
11	0	5.2a	4.9a	Yes (P = 0.0118)
	24	3.9b	3.8ab	
	48	3.5b	3.5b	
12	0	4.6a	4.7a	Yes (P = 0.0283)
	24	4ab	4.1ab	
	48	3.4b	3.4b	

*Sensory score: 0 = no odor; 10 = high odor

**Means w/in groups w/different letter = significantly different at P = 0.05 using an LSD test.

Factory conditioning trials were performed in the same manner as those at Research (Methods).

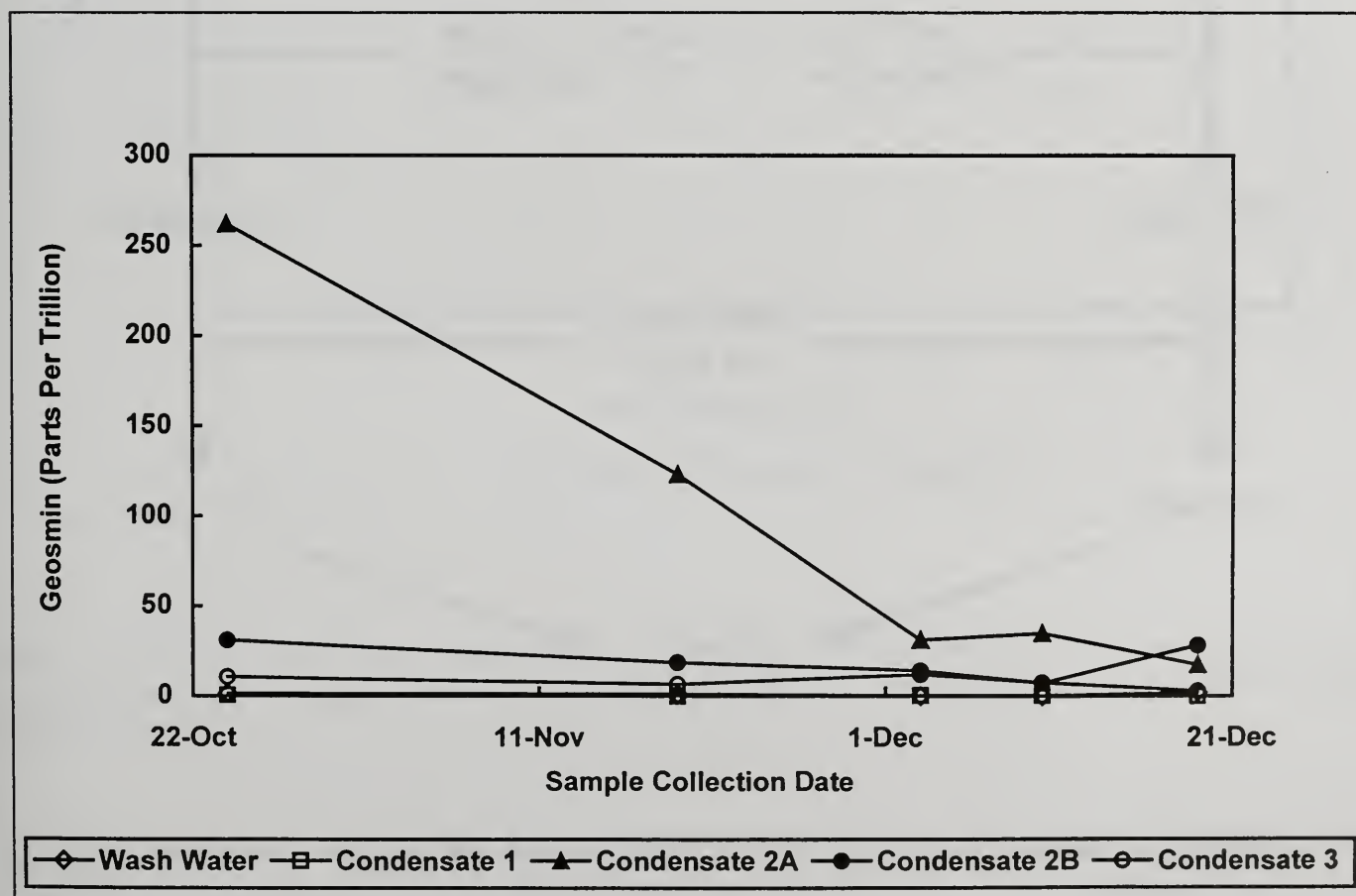


Figure 1. Geosmin in Factory "A" condensates.

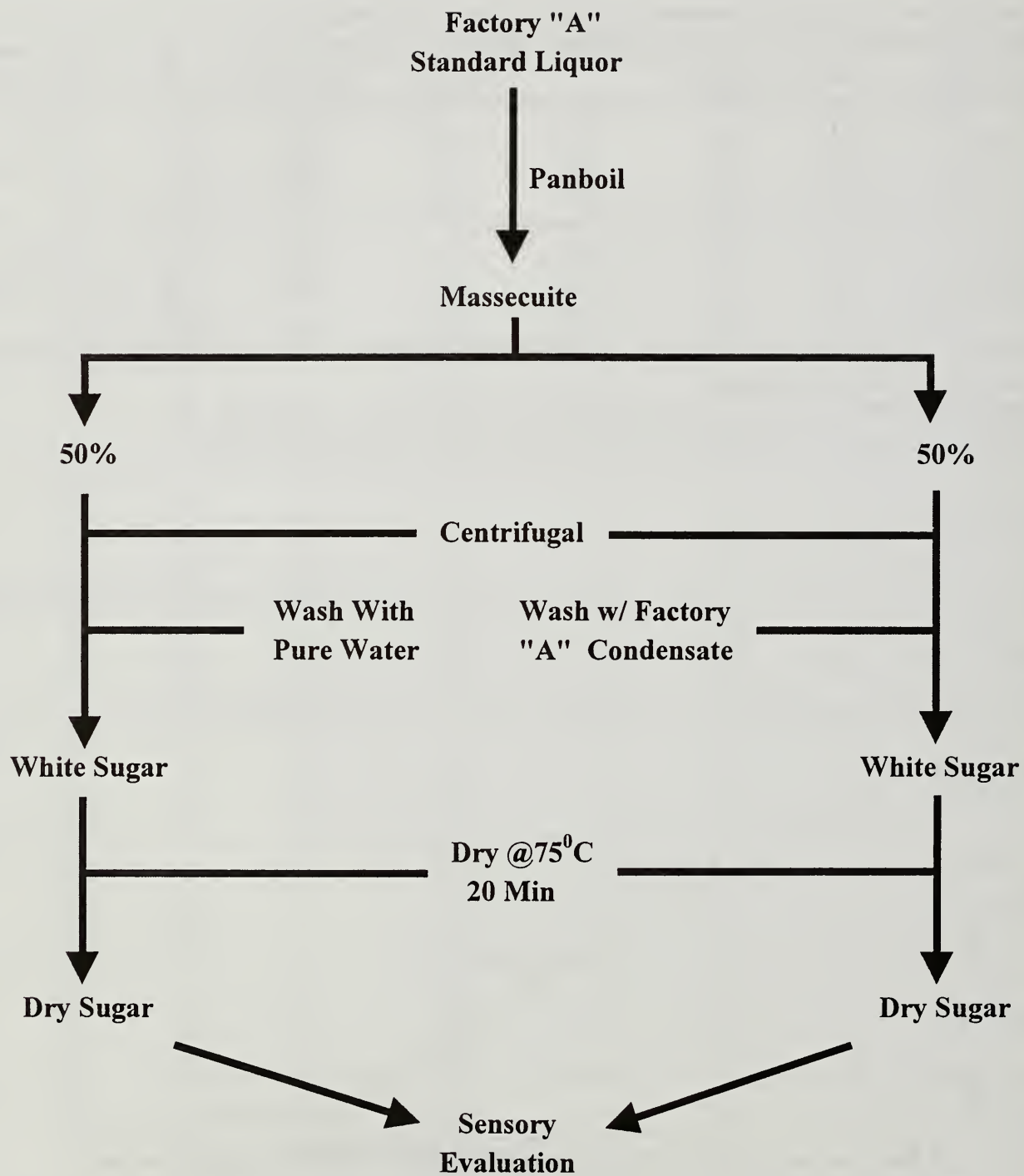
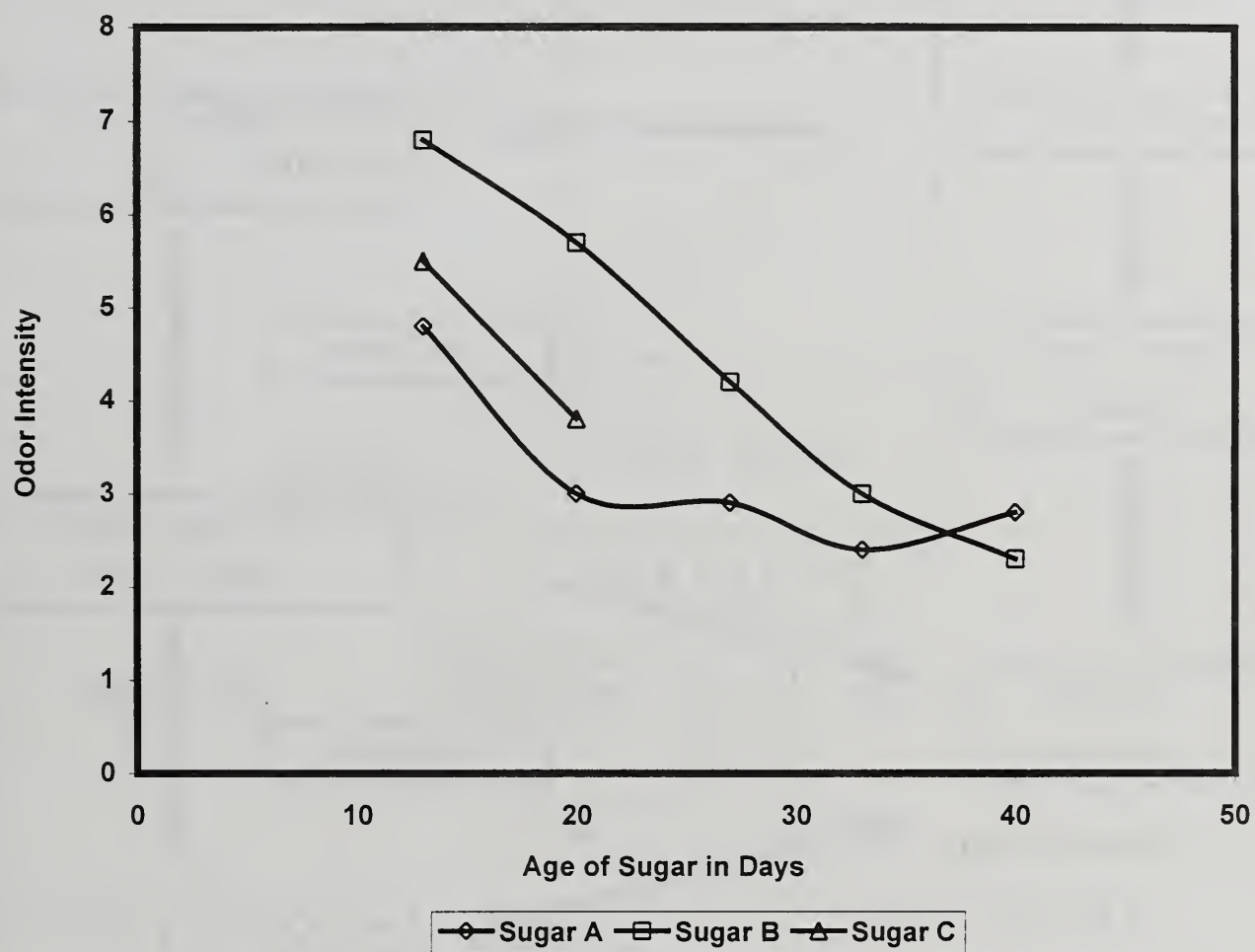


Figure 2. Scheme for testing effect of wash water quality on sugar odor.



Sugar was collected on different dates during the autumn of 1996 and stored in plastic bags @ room temperature at ACS' Research Center. At weekly intervals, the sugar was evaluated for odor by sensory panel (Methods).

Figure 3. Odor intensity of sugar as a function of time.

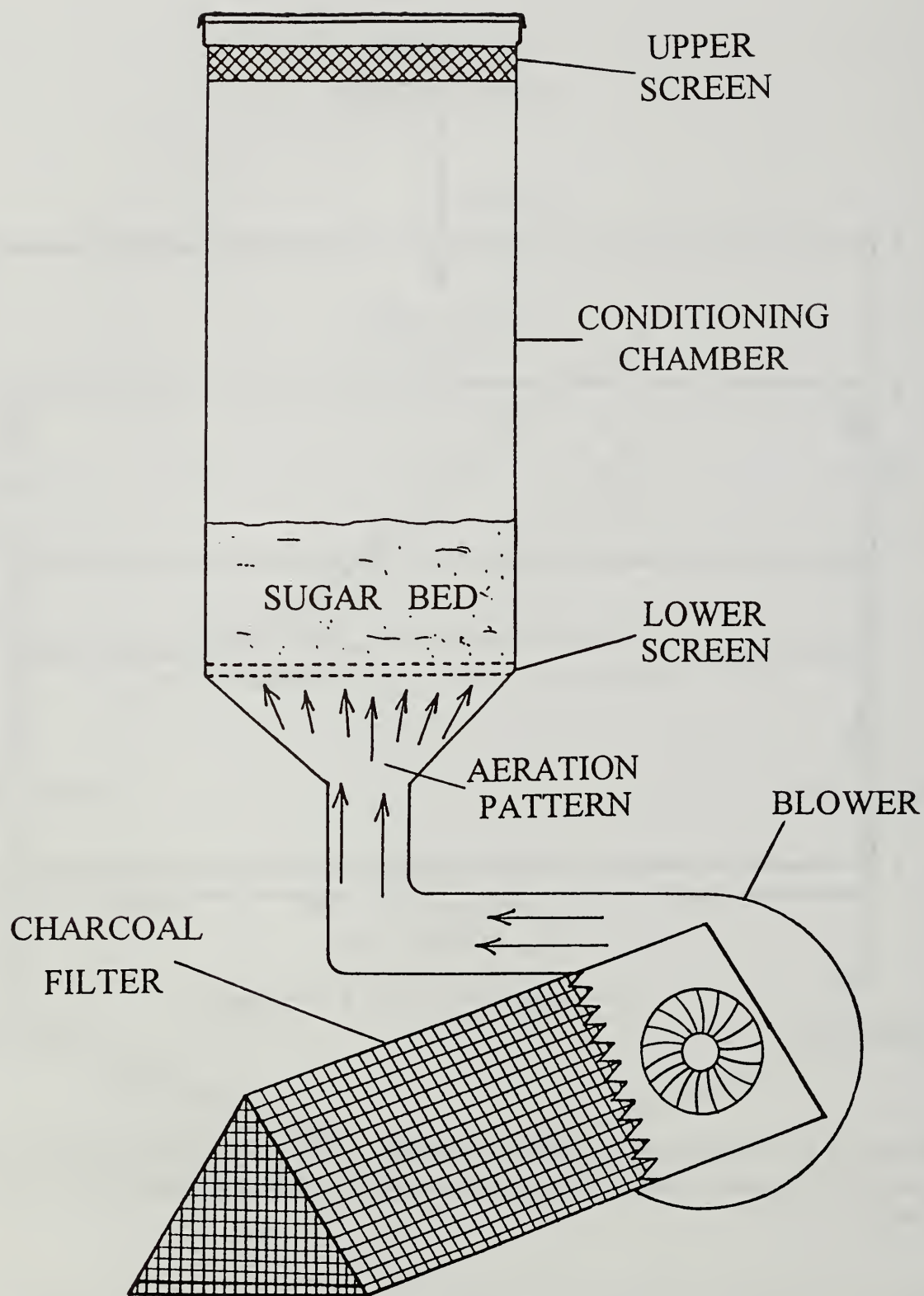


Figure 4. Schematic diagram of pilot-scale sugar conditioning bin.

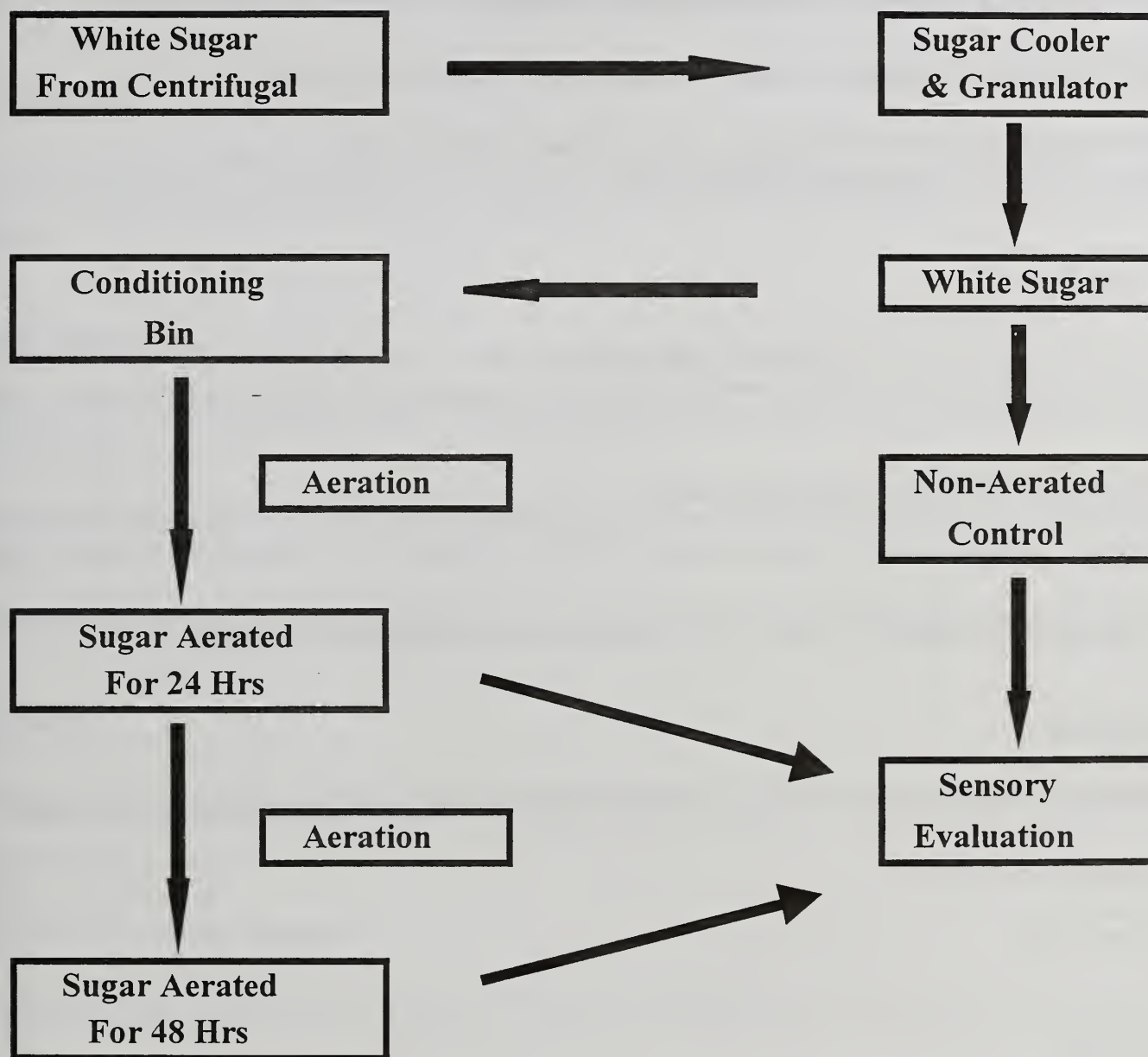


Figure 5. Schematic diagram of sugar conditioning process.

S.P.R.I.

POSTER

GRAIN SIZE DETERMINATION OF WHITE SUGARS

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ABSTRACT

Grain size determination of white sugars is traditionally measured in a series of size-calibrated sieves. Noise generated by the sieve holders during separation requires precautions for protection of employees' hearing.

STANDARD ANALYSIS FOR GRAIN SIZE

PURPOSE

To determine the percentage by weight of the various sugar granulations in the sample.

APPARATUS

A nest of sieves (U.S. or Tyler) with pan and lid Ro-Tap sieve shaker or other appropriate type shaker.

Balance sensitive to 0.1g

Sample splitter

Fine-bristle brush

REAGENTS

None

PROCEDURE

Divide the sample in the sample splitter sufficient times to quarter it to slightly over 100g. Weigh out 100g and place on top screen of screen nest equipped with pan and lid. Place nest in sieve shaker and tap for 5 minutes. After tapping, put the retained sugar from each screen in the sieve nest and record the weight as the percentage retained on that screen.

CALCULATION

Record the weight retained on each sieve as the percentage retained.

REFERENCE

Methods of Analysis of White Sugar by U.S. National Committee on Sugar Analysis. pp. 288-302. In Sugar: A User's Guide to Sucrose. (1990). Eds. N.L. Pennington and C.W. Baker Van Nostrand. Reinhold, N.Y., 330 pp.

EXPERIMENTAL

Samples (40) of white granulated sugar and their analysis by the standard sieve technique were obtained from Refinery A.

Samples were analyzed on a similar set of standardized U.S. sieves, on an automated Retsch analytical sieve shaker. Model AS2000 (Retsch gmbH and Co. D-42781. Haan, Germany. Brinkman and Co. in U.S.A.)

Various amplitude, time and interval settings were tested to achieve results most similar to the standard test.

RESULTS

Settings for Model AS2000. Optimum settings were found to be, for 10 sieve pans from 20 to 140 mesh:

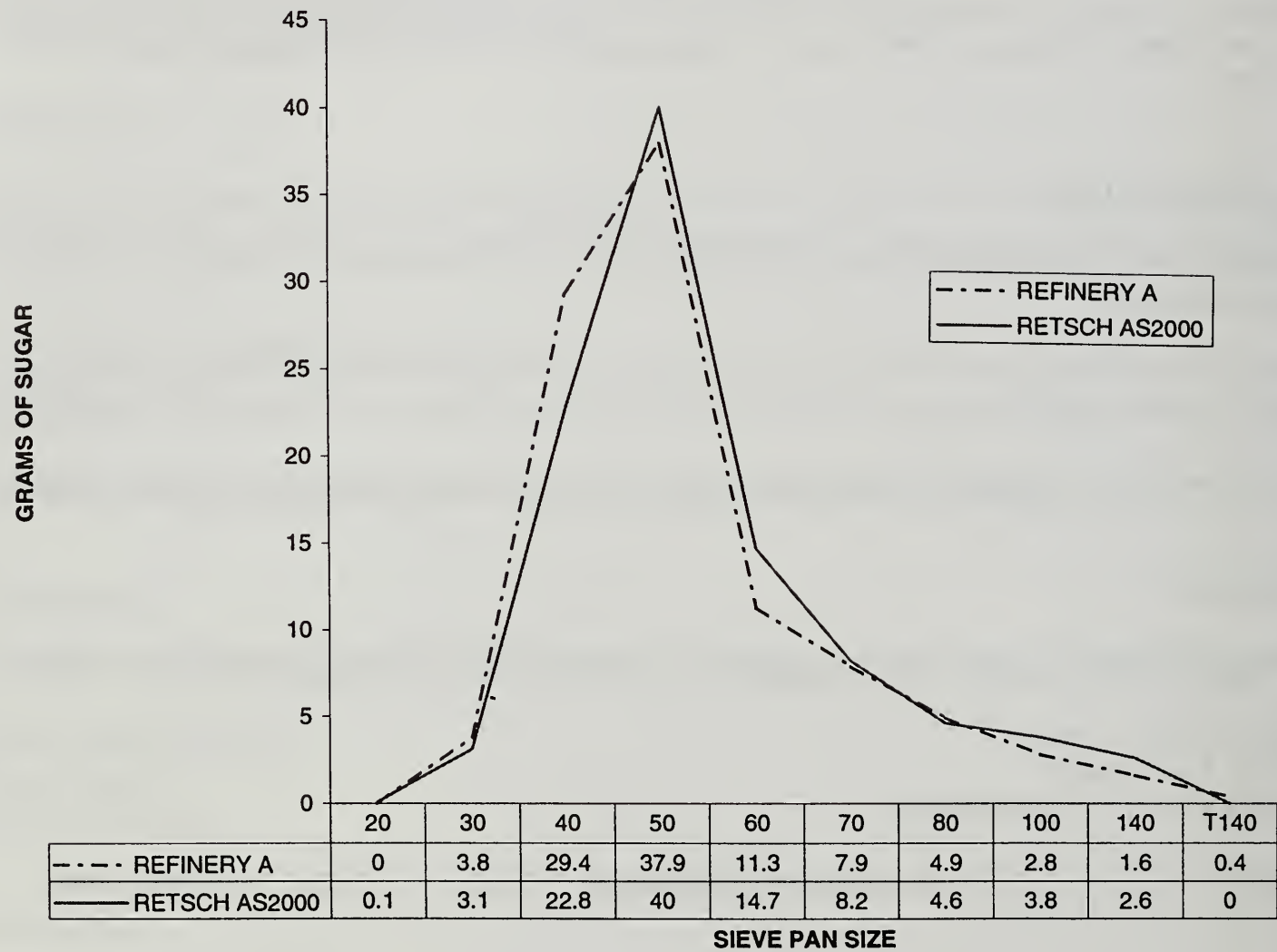
AMPLITUDE: 0.40mm

TIME: 3 to 5 minutes

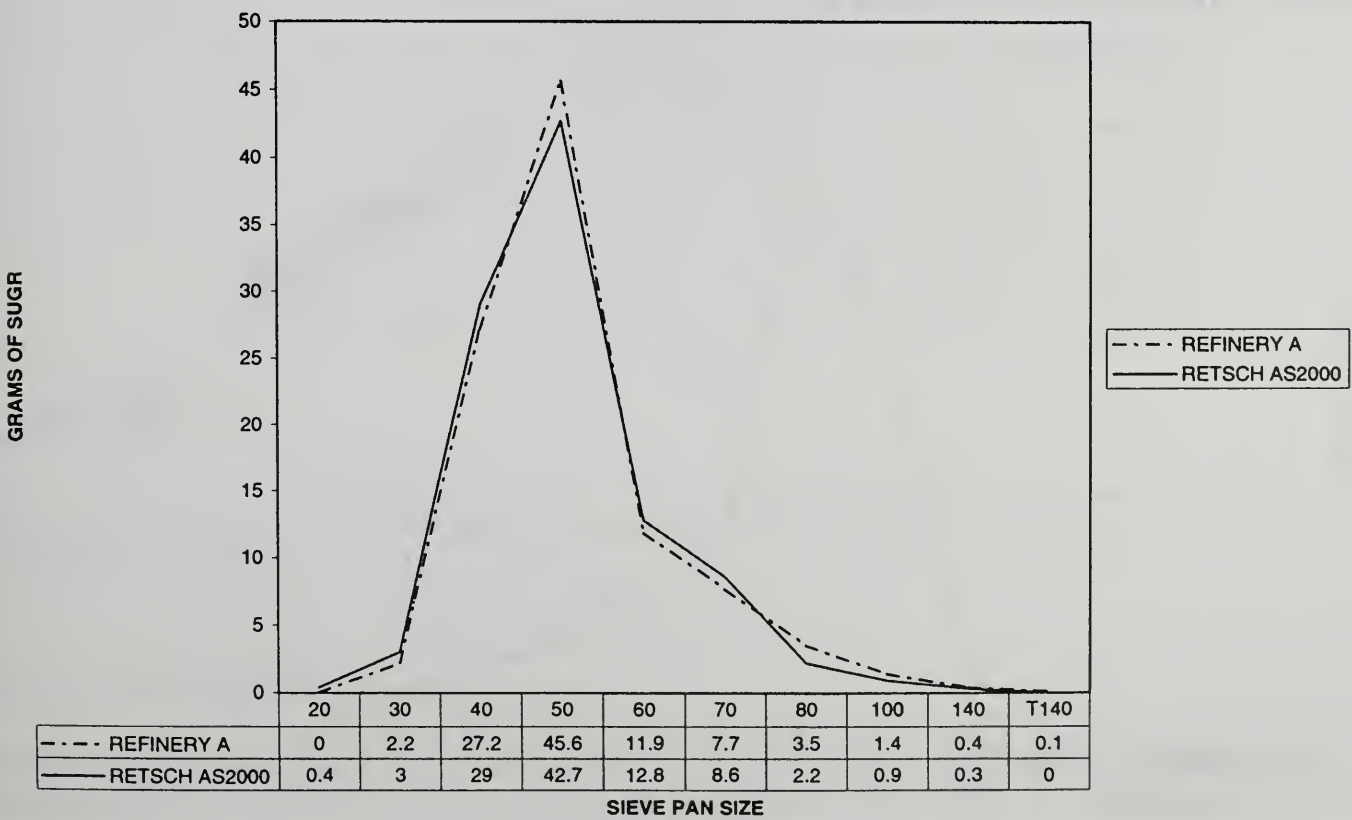
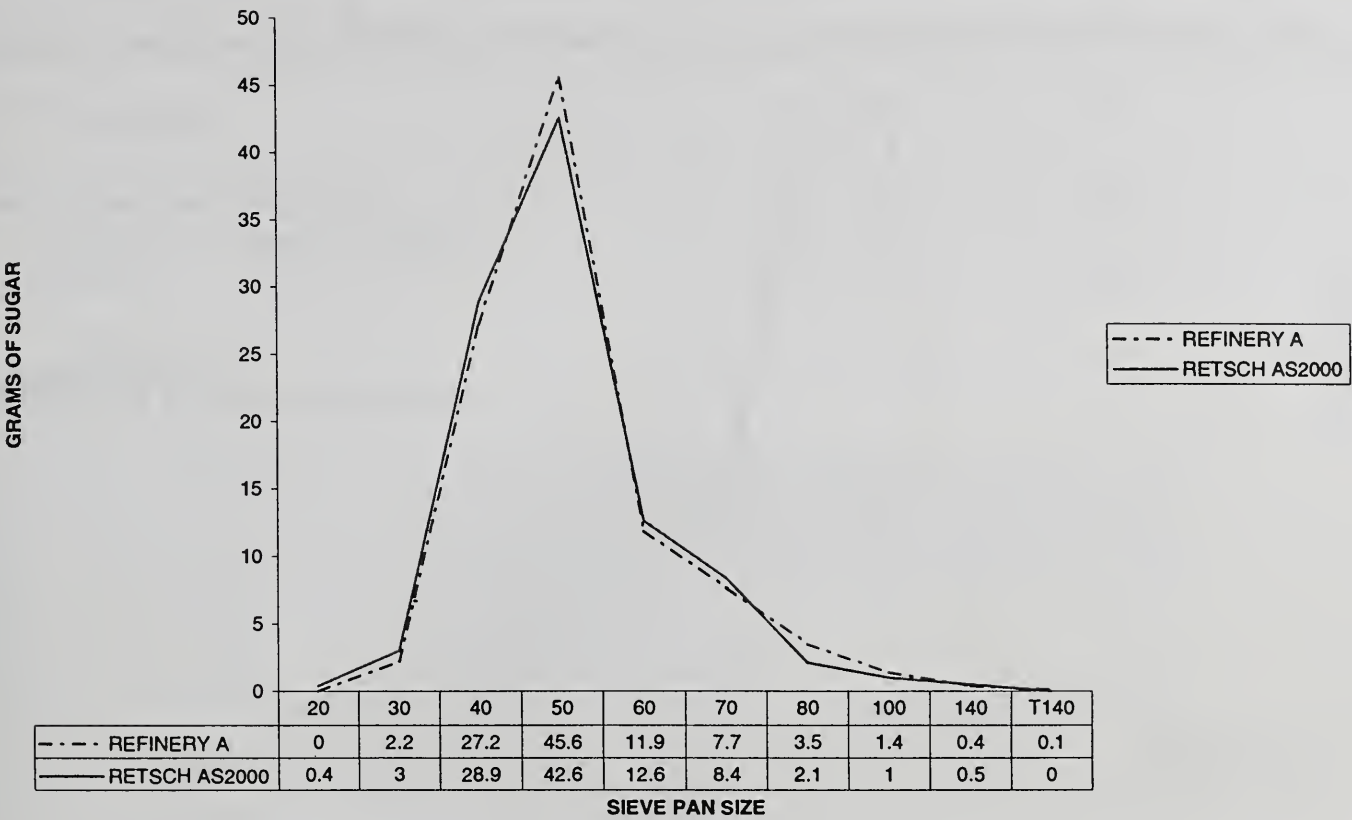
INTERVAL: 10 second intervals

Distribution of grain size for several sugars, comparing the standard test to Retsch, are shown below.

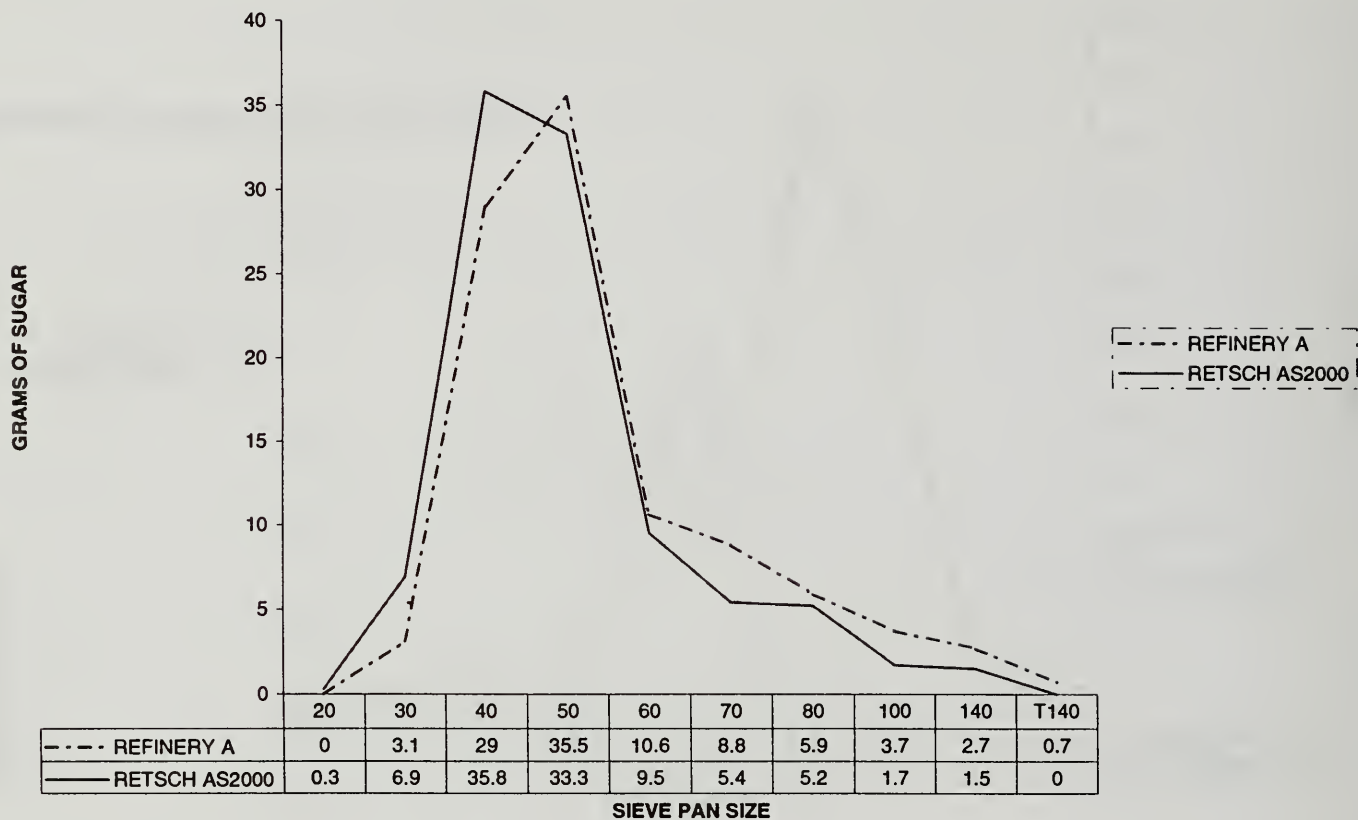
REFINERY WHITE SUGAR SAMPLE



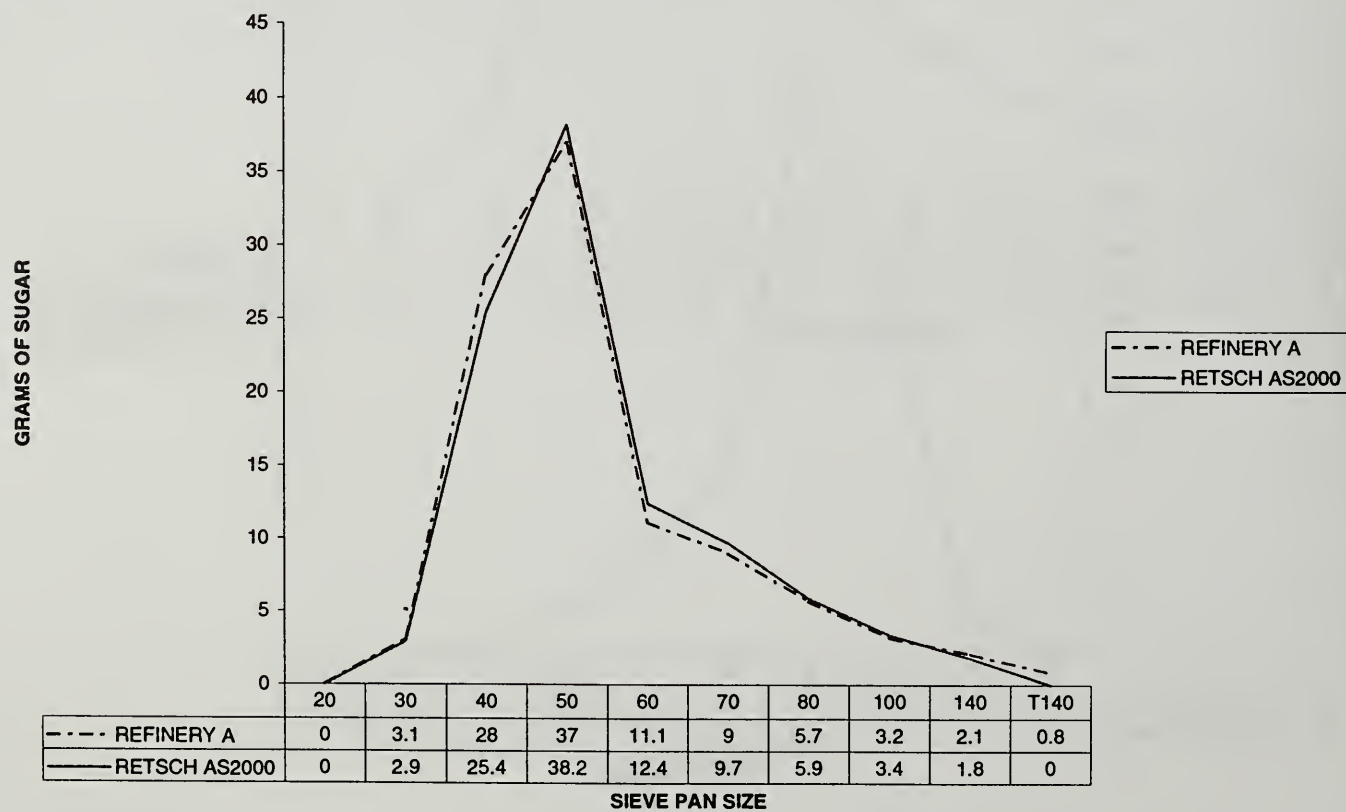
DUPLICATE RUN



REFINERY WHITE SUGAR SAMPLE



REFINERY WHITE SUGAR SAMPLE



COMPARISON OF STANDARD SIEVE WITH RETSCH

The graph below shows the correlation for Retsch vs. Refinery A standard test. The correlation equation is:

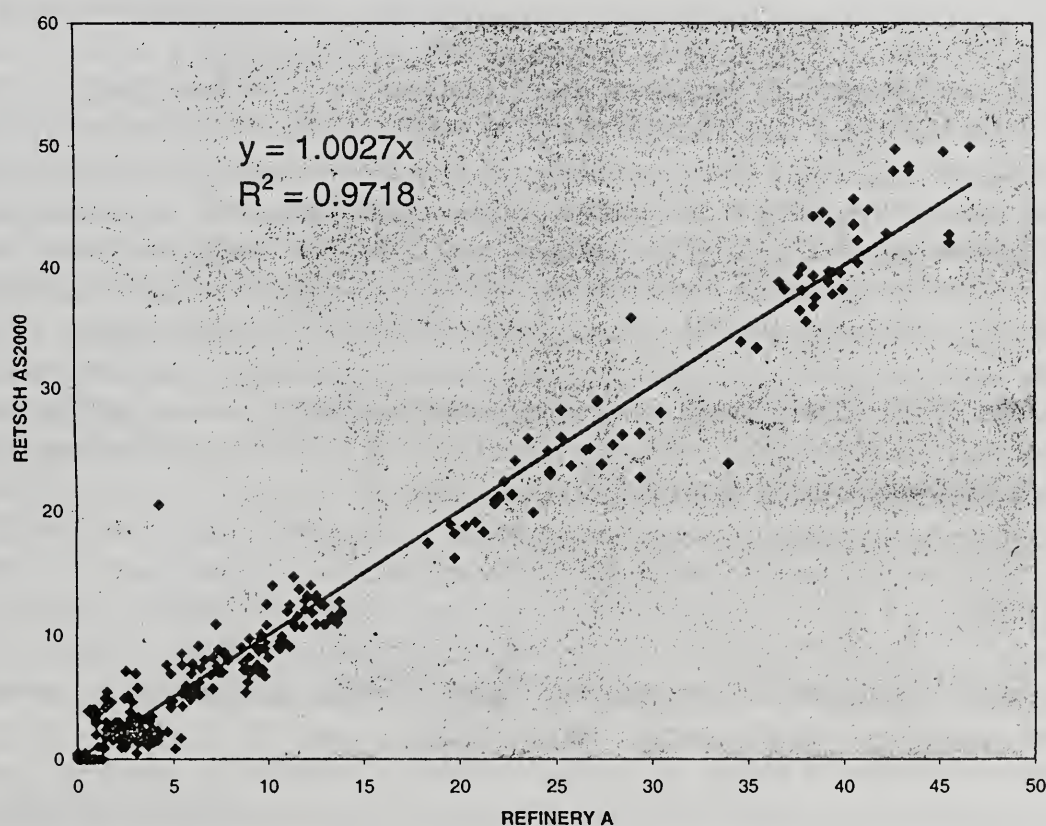
$$Y = 1.0077X - 0.1251$$

where Y=Retsch weight percent

X=Refinery A's weight percent

and $R^2 = 0.972$

REFINERY A VS RETSCH AS2000



CONCLUSION

The new quiet sieving system by Retsch gives good equivalence (0.97 correlation) to the regular sieve test.

ACKNOWLEDGMENTS

Grateful thanks are extended to Dr. Bryan Vinyard of Southern Regional Research Center for statistical treatment of data.

POSTER

MONITORING BEET SUGAR EVAPORATOR SYRUP INVERT AND SUCROSE COMPOSITION BY ION CHROMATOGRAPHY

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ABSTRACT

Samples of beet sugar evaporator syrups were received from a sponsoring corporation in the sugarbeet processing industry to examine concentrations of sucrose and invert syrup across the six stages of their evaporators from Thin Juice to Thick Juice. Sample dilution was a particularly difficult choice to make so that replicate runs could be made to estimate both total sucrose as well as invert sugar forming across the battery of evaporator pans. In general, based on a °Brix solids dilution, a 1% solution of solids from the syrup was again diluted 1 to 100 to afford a total injection in the low parts per million range. It was found to be helpful that after an isocratic 100 mM elution of the sugars, a short 200 mM sodium hydroxide wash of the column was introduced during the gradient program. This stabilized the column separation characteristics. Day to day operation of the sugarbeet factory on the selected dates of sampling shows some variations in factory mechanical controls. The invert sugar and sucrose are related to total sugar mass balance in the syrup concentration process. Unknown peaks in the evaporator syrups seem to follow no particular pattern during the concentration. Although only limited pol values were available for comparison, sucrose purity by ion chromatographic determination of sucrose concentration accurately reflects sucrose purity and its degradation to invert.

INTRODUCTION

Sugar losses during concentration in the evaporator stages have been studied from the point of view of pol, purity, pH, and color by several groups. Most recently, work of Eggleston (2,3) reported ion chromatographic monitoring of sugars as another variable of changes in chemistry across sugarbeet evaporators. A significant result of the work by Eggleston and coworkers is the identification of important marker compounds across the battery of evaporators which signal degradation of sugar due to maladjustments of operating parameters such as pH, residence heating, and concentration factors. Recently, Sargent and coworkers (5) described thick juice degradation during storage due to bacterial growth. However, less is understood about sugar mass balance in the high temperature, dynamic chemistry of sugarbeet processing.

OBJECTIVE

The objective of the present paper was to determine total sugar, sucrose, and invert mass balance across the entire train of sugarbeet evaporators at the exit points of concentrated syrups as variables changed

throughout an entire working season. The samples compared in the present study are representative of a typical beet sugar factory.

EXPERIMENTAL

Samples (seven) over a six month period were taken at the respective exit ports of the sugarbeet factory evaporators and shipped frozen to Sugar Processing Research Institute, Incorporated, New Orleans, LA, USA. Careful storage and thawing of the evaporator syrup was carried out after receipt.

Sugar analysis methods were taken from the International Commission for Uniform Methods of Sugar Analysis *Methods Book*, April, 1994. Analyses performed by ICUMSA methods (4) were pol, refractometer Brix, apparent purity, conductivity ash, and pH.

High performance liquid chromatography of sucrose, glucose, and fructose for the beet sugar factory evaporator samples and sugarbeet ion exclusion separator samples was performed on a Dionex PAC-1 column with pulsed amperometric detection using the Dionex AI-450 integrating computer program. The column was run at ambient temperature with 100 mM carbonate free sodium hydroxide in purified water (18 megohm-cm resistivity) under helium sparging and flow rate of 1.0 ml/min with a 200 mM sodium hydroxide wash at the end of the run and re-equilibration to 100 mM eluent before the next run (turnaround time per sample, 21 minutes). Samples were filtered through a 0.45 μ M nylon filter prior to injection. The evaporator syrup ion chromatography was effected as in the AOAC Methods (1). Calibration responses with 2-amino-2-deoxy-glucose as internal standard at 5 ppm were run repeatedly over the entire period of sampling. External standards of 0.5, 1, 2, 3, 4, and 5 ppm were employed for glucose and fructose, respectively, and sucrose at 1, 3, 7, 10, 15, and 20 ppm to guarantee a linear response. Evaporator syrup samples were diluted to optimum linear response range on the ion chromatograph pulsed amperometric detector, namely in the range of less than 5 parts per million of glucose and fructose and less than 20 ppm for sucrose. From Brix readings of solids enough syrup was weighed to make 1 gram syrup solids in 100 ml solution. This solution was further diluted 0.1 ml to 100 ml to provide solution for filtration through a 0.45 μ M membrane prior to injection of 20 μ L onto the column.

RESULTS AND DISCUSSION

Table 1. Sugar concentrations of beet sugar factory evaporator syrups.

In this table the average invert sugar, sucrose, and total sugar are compared to Brix, pol, purity, and conductivity ash over the entire evaporator series for the exit syrups. Although the standard deviations are rather broad (± 3 to 8%), these averages of values still represent the whole process for more than 6 months of sampling period. Regression analyses of each of the average in the columns against the refractometer Brix, pol, and conductivity ash gave high correlations ($r^2 = 0.95$).

Figure 1. Average total sugars in evaporation stages 1996.

In Figure 1, three typical sampling dates are compared to show variations of sugars across the evaporators. Allowing for processing and mixing periods the reproducibility of the system is quite close. These data are included in the values of Table 1.

Figure 2. Average pH in evaporation stages 1997.

The pH was held at between pH 8.8 and 9.2 in this factory. The pH is lower in the thin juice and gradually rises and falls to the Exit Syrup 4 stage. The pH rises again in the thick juice.

Figure 3. Average pol in evaporation stages 1997.

The pol averaged over a factory period of more than a month is maintained at each exit stage in fairly close values. The rise in pol levels off at Exit Stage 4 and the Thick Juice. Pol and Brix parallel each other also closely as do sucrose and total sugars as illustrated in Table 1.

Figure 4. Average conductivity ash in evaporation stages 1997.

The ash concentration reflects the concentration process overall. The highest concentrations of ash are near the end of evaporation and are reflected in the highest Brix readings there.

Figure 5. Sugar concentrations of beet sugar evaporators.

The sucrose and total sugars parallel each other throughout the evaporation process. However, the invert sugars go through a maximum in the Exit 4 Syrup and then decline in the thick juice. The rise in invert sugar may also be related to more concentrated ash and local pH changes that are more conducive to sucrose hydrolysis.

CONCLUSIONS

- The ion chromatography determined reasonable mass balances of the sugars and reproducibility. The twelve sample dates examined show similar trends in terms of rates of change of sugar concentration as shown on Figure 1 and rates determined from slopes made by regression of the concentrations.

- Percent purity of the final thick juice follows that of the earlier samplings. Invert sugar concentration drops off from Exit Syrup 4 to Thick Juice by almost half while sucrose and total sugars rise somewhat from Exit Syrup 4 to Thick Juice as continuing evaporation occurs during cooling.

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Table 1. Sugar concentrations of beet sugar factory evaporator syrups.

SUGAR CONCENTRATION OF BEET SUGAR FACTORY EVAPORATOR SYRUPS								
	Glucose average all 7	Fructose average all 7	Sucrose average all 7	Total sugars average all 7	Average °Brix-tc evaporator syrups	Average pol	Average percent conductivity ash	Purity percent
Thin juice	0.07	0.22	15.51	15.80	17.06	16	0.27	93.76
Evap syrup 1	0.08	0.15	20.04	20.26	21.06	19.42	0.35	92.22
Evap syrup 2A	0.06	0.10	22.95	23.11	27.72	26.17	0.42	94.41
Evap syrup 2B	0.13	0.32	31.88	32.33	33.96	31.93	0.46	94.03
Evap syrup 3A	0.20	0.44	44.67	45.31	51.24	48.78	0.58	95.2
Evap syrup 3B	0.24	0.37	53.95	54.56	58.27	56.36	0.61	94.99
Evap syrup 4	0.22	0.78	66.05	67.05	71.37	67.19	0.67	94.14
Thick juice	0.22	0.33	67.53	68.08	72.42	68.19	0.67	94.16

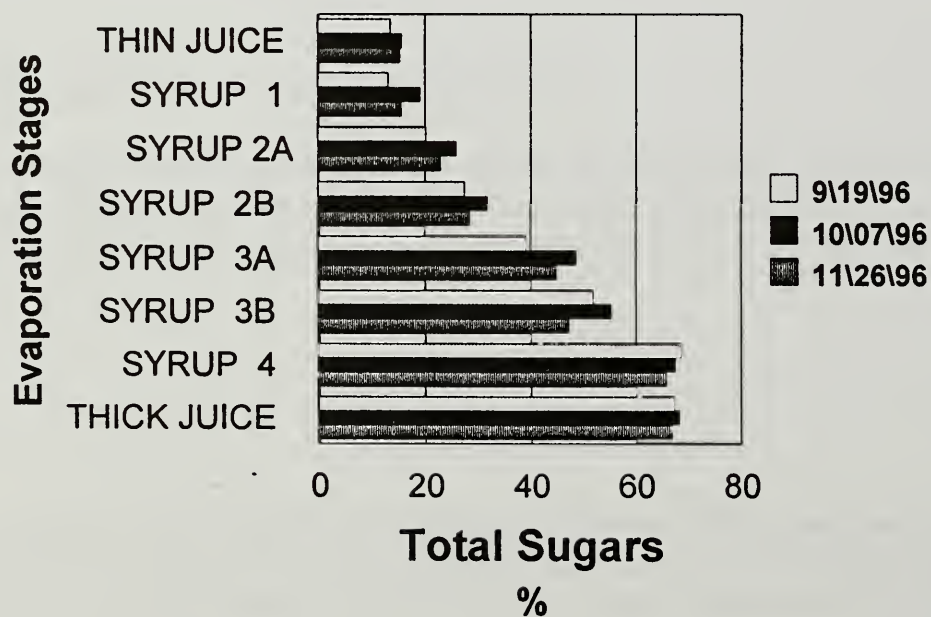


Figure 1. Average total sugars in evaporation stages 1996.

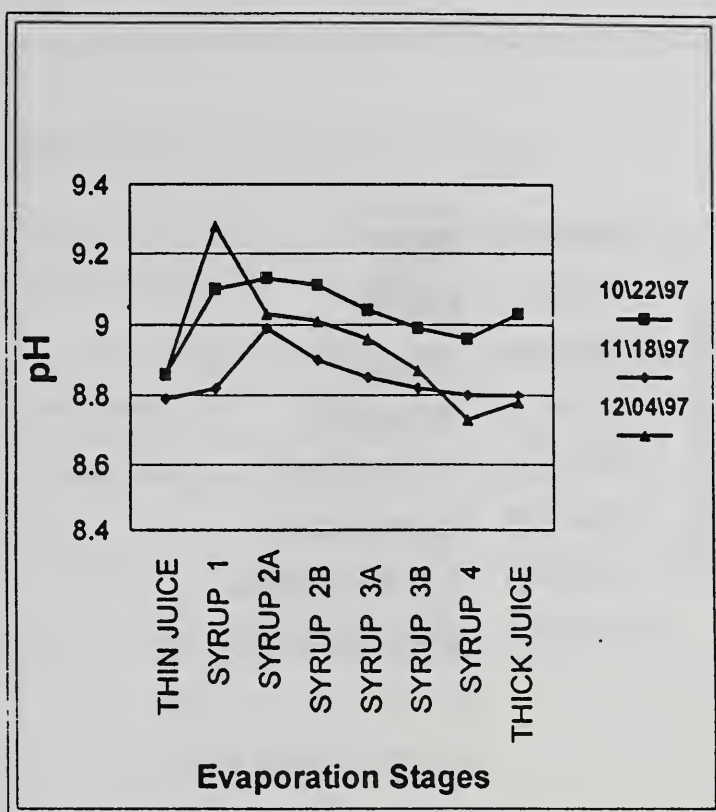


Figure 2. Average pH in evaporation stages 1997.

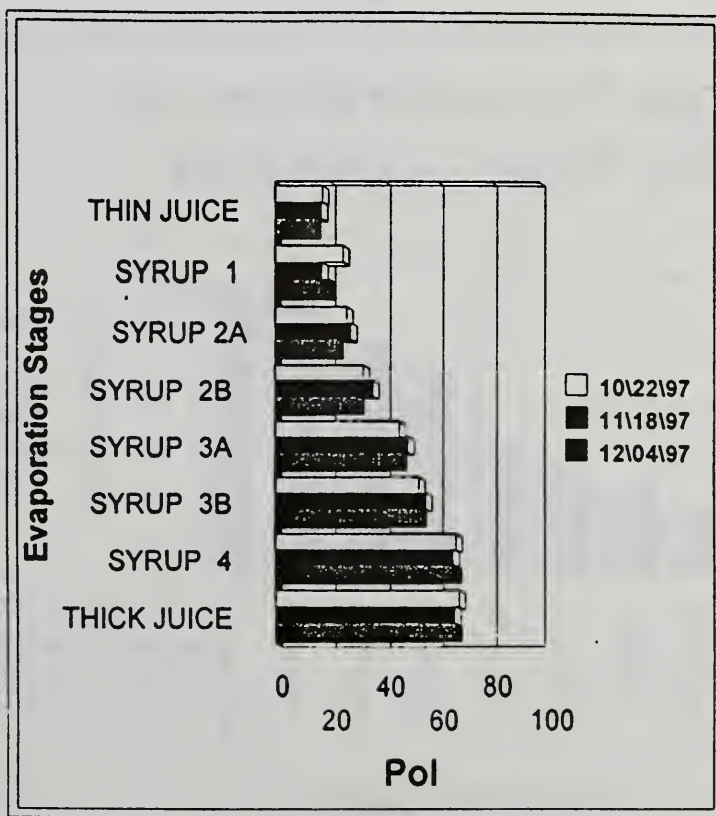


Figure 3. Average pol in evaporation stages 1997.

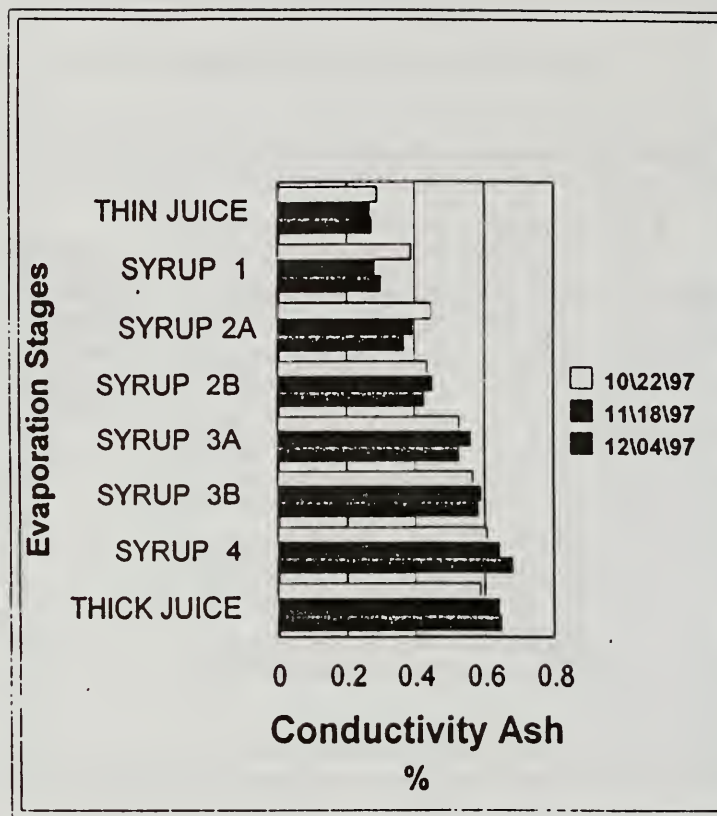


Figure 4. Average conductivity ash in evaporation stages 1997.

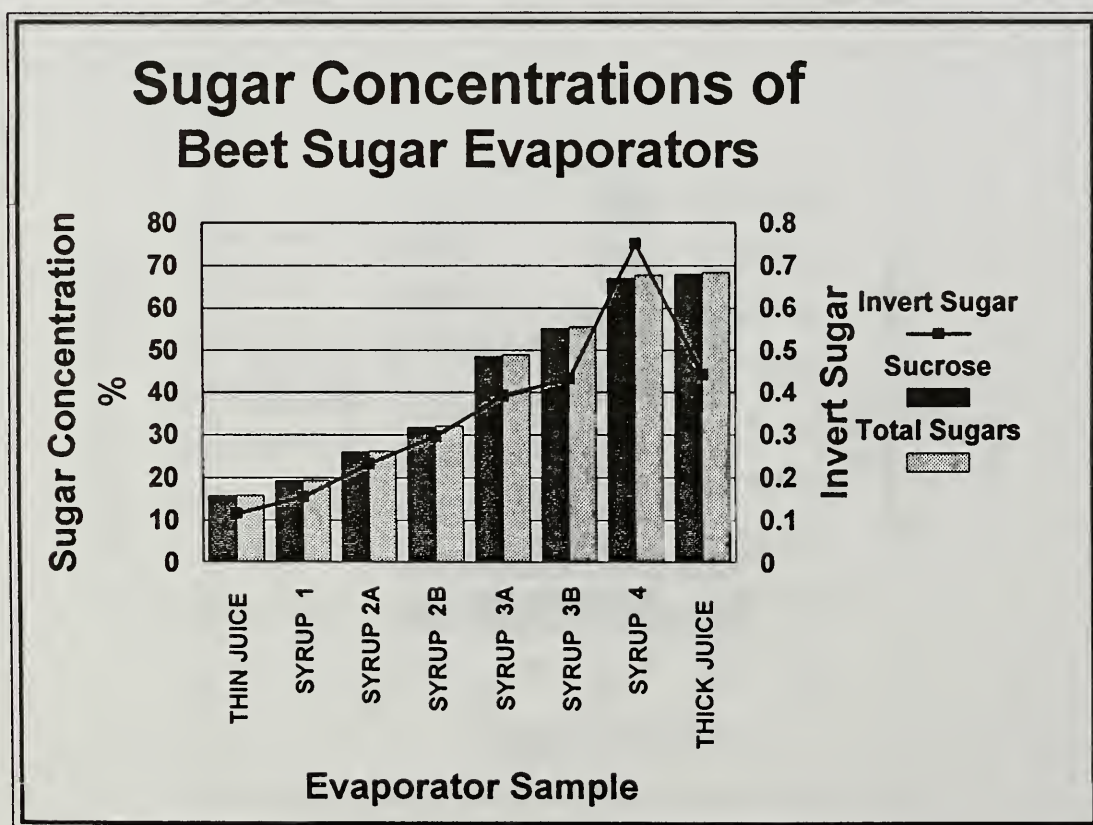


Figure 5. Sugar concentrations of beet sugar evaporators.

POSTER

EFFECT OF CANE QUALITY ON SUGAR PRODUCTION

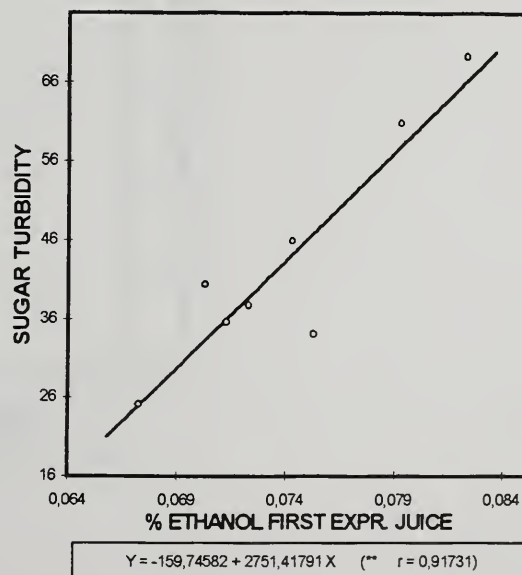
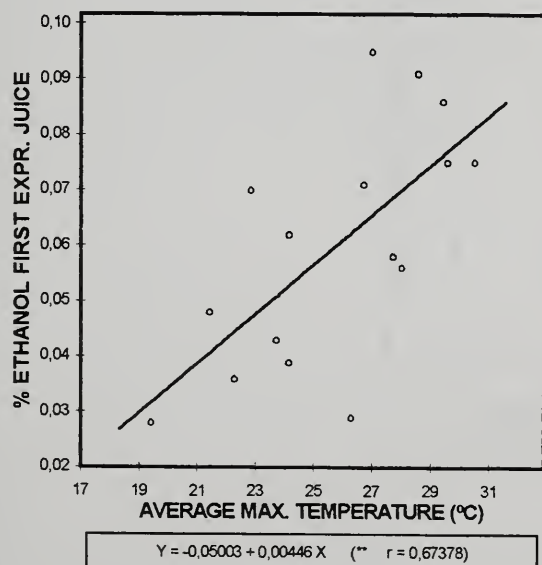
Henrique V. Amorim, Antonio J. Oliveira, Luiz F. L. F. Silva and Alexandre Godoy, Fermentec S/C Ltda and University of São Paulo, Piracicaba, S. P., Brazil

It is well known that differences or changes in the environment in harvesting and in cane transportation can have an important impact on sugar production and quality. In this study, regression analysis was carried out on weekly averages of several environmental parameters against sugar quality. The parameters tested included ambient temperature, relative humidity, mud, and time from burn to crush. Quality factors included ethanol in first juice, turbidity, *Bacillus* in cane juice, black points, dextran, sulfite usage in clarification, syrup color, level of insolubles in sugar, filterability, and reducing sugars.

The most significant correlations are discussed.

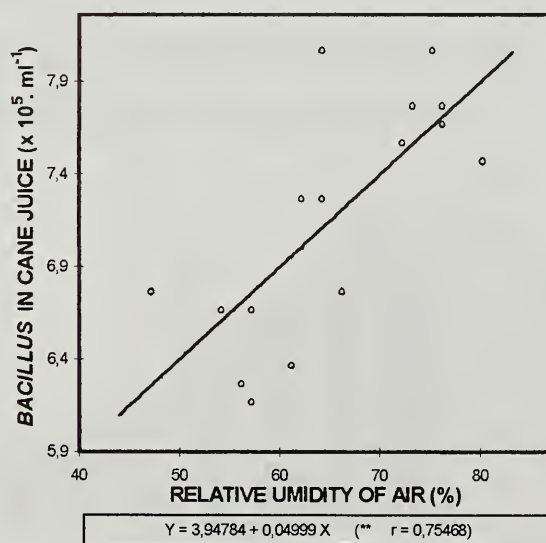
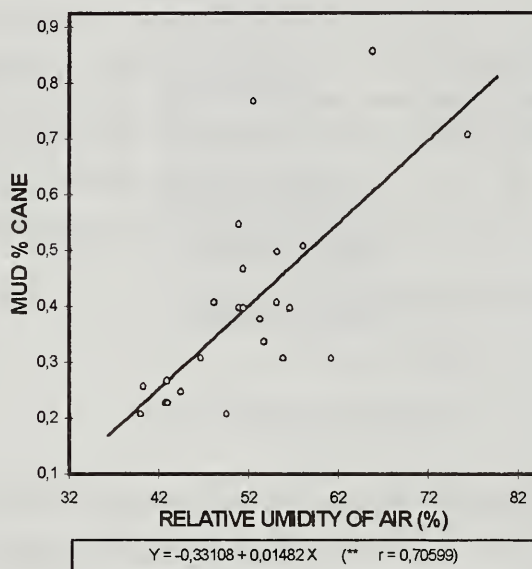
RESULTS

The ambient temperature in the week that the cane was harvested and crushed was positively correlated with ethanol in first expressed juice ($r=0.67$) and the ethanol in the first juice correlated positively with sugar turbidity ($r=0.91$).

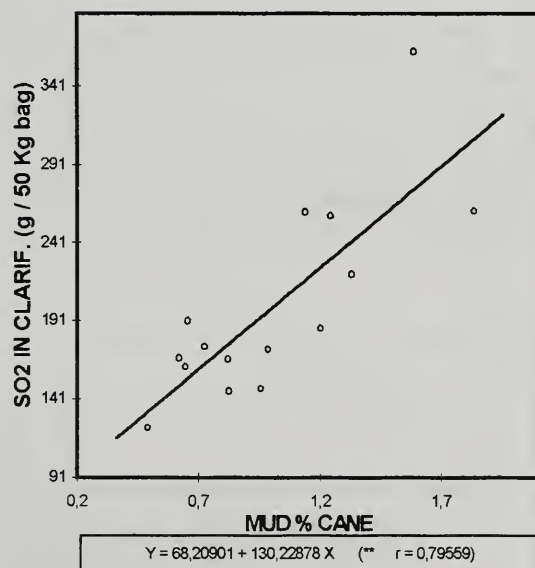
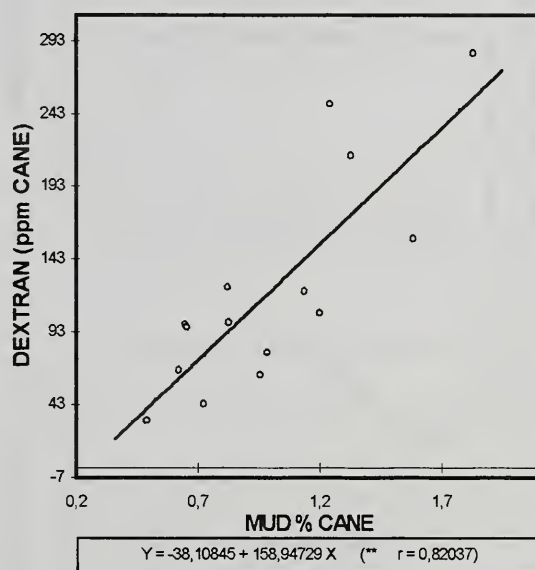


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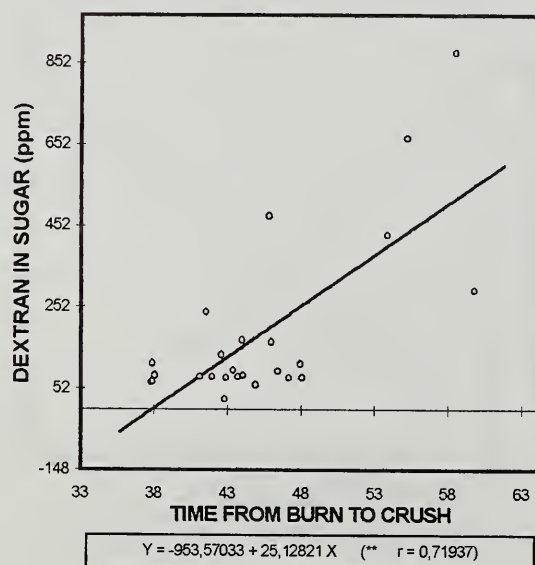
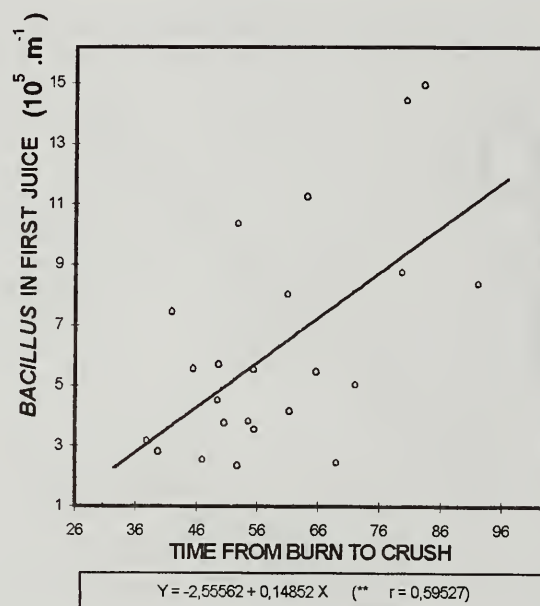
The relative humidity of the air gave a positive and strong correlation with soil (mud) in cane ($r=0.70$), and also with *Bacillus* in cane juice ($r=0.75$).

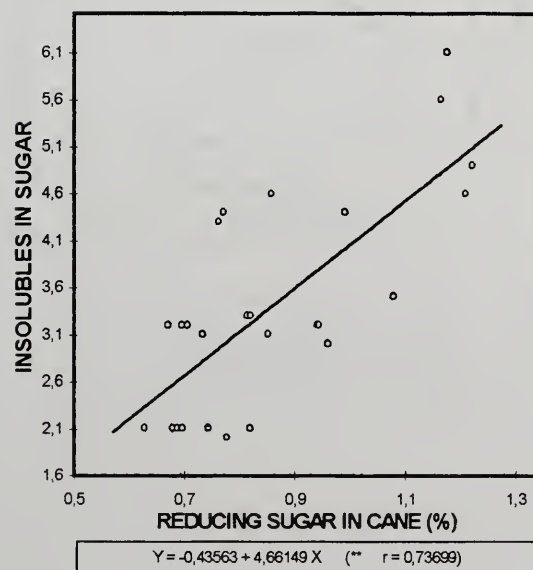
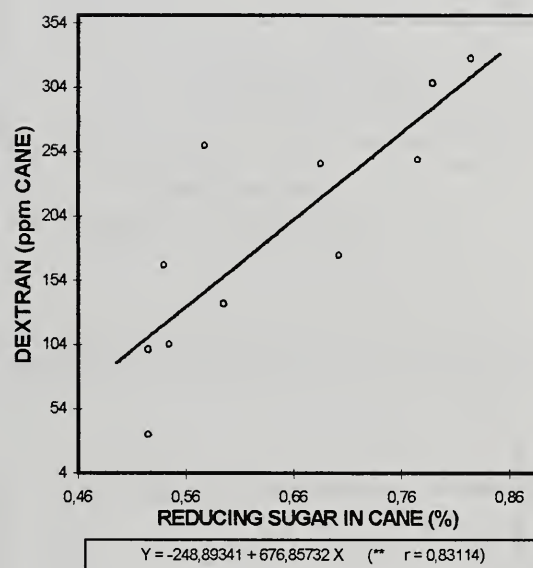
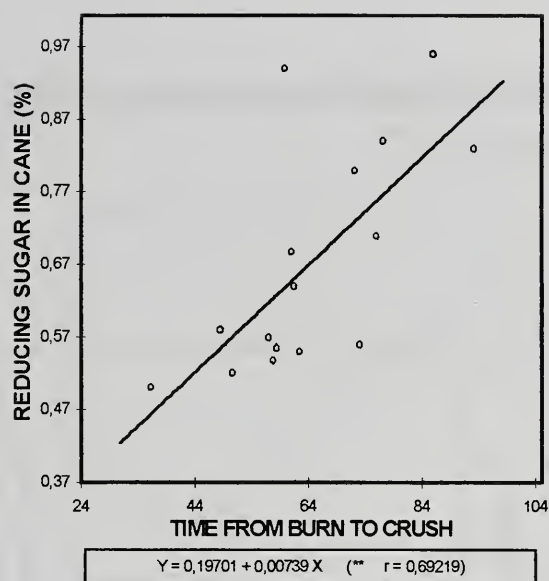


Mud (soil) gave a positive correlation with dextran in cane ($r=0.82$), also a positive correlation with the use of sulfite in clarification ($r=0.79$); also a positive correlation with syrup color ($r=0.69$) and also with the level of insolubles in sugar ($r=0.55$).



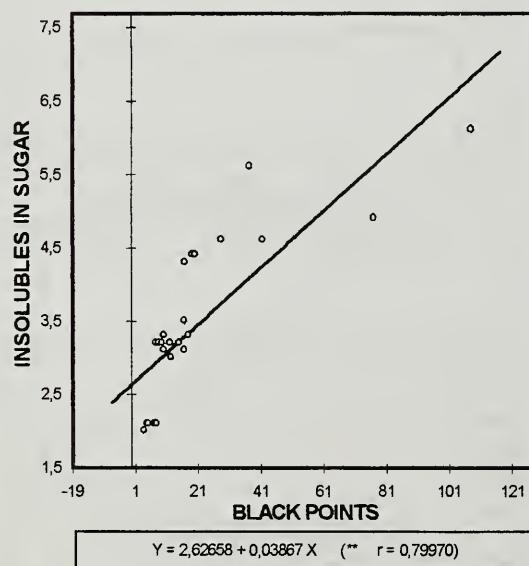
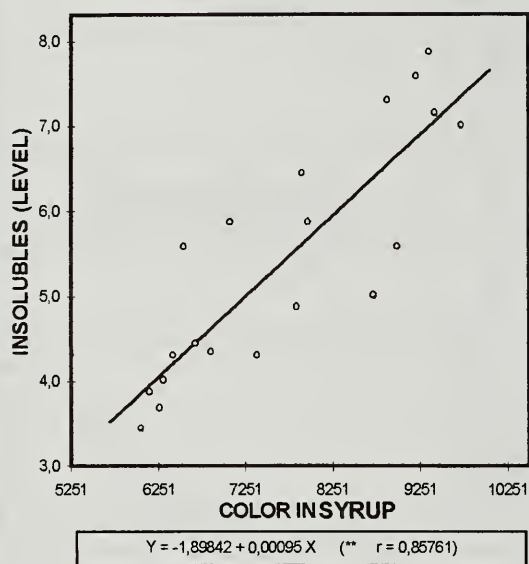
The time from burn/cut to crush was correlated positively with *Bacillus* in the first expressed juice ($r=0.59$), positively also with dextran in sugar ($r=0.72$), positively with reducing sugar in cane ($r=0.69$). Reducing sugar in cane was correlated positively with dextran in cane ($r=0.83$) and also positively with insolubles in sugar ($r=0.74$).



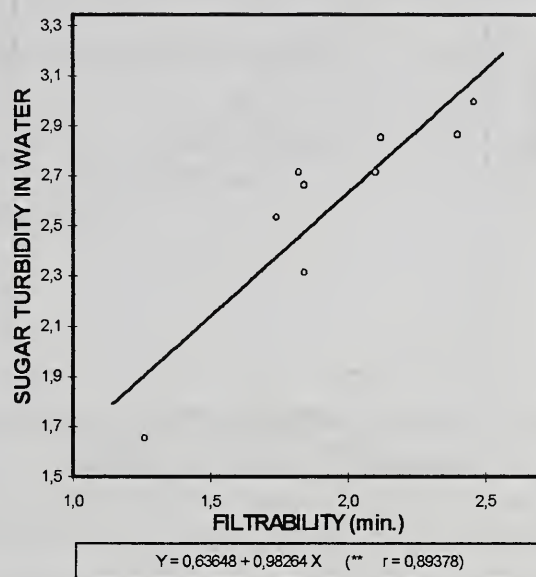


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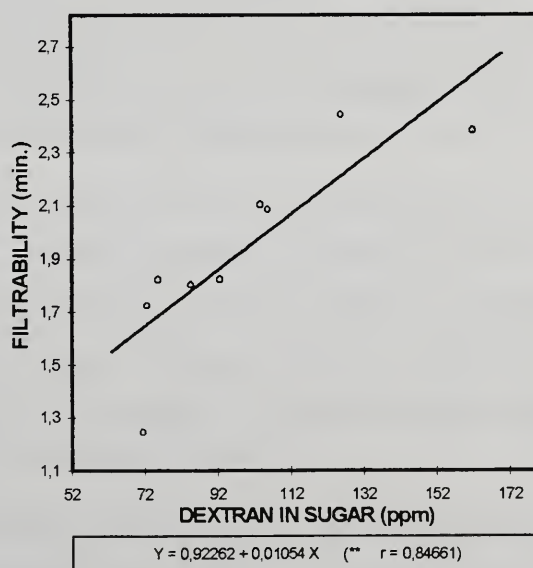
Color in syrup was correlated positively with insolubles in sugar ($r=0.85$). Black points and insolubles in sugar also had a positive correlation ($r=0.79$).



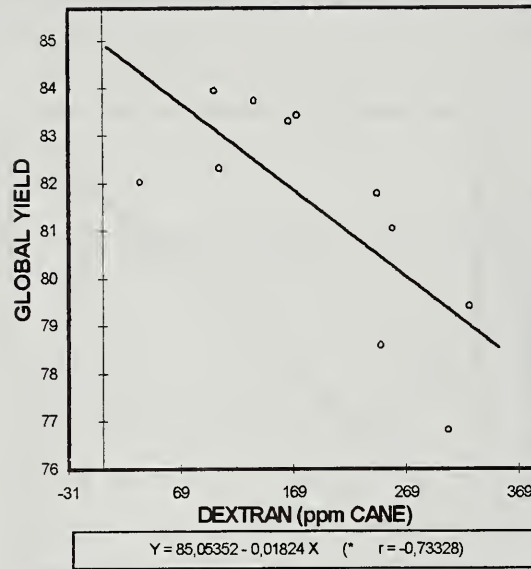
Filterability (increasing time of filtration means lower filterability) in sugar was correlated positively with sugar turbidity (water) ($r=0.89$).



Dextran in sugar was correlated positively with filterability ($r=0.84$) and negatively with global yield in the plant ($r=-0.73$).



S.P.R.I.



CONCLUSION

Regression analysis can be used to obtain an idea of what factors are impacting the process and product quality, and can be used to quantify losses in the plant. However, care should be taken to avoid misleading interpretation. Multiple regression analysis could also be used, again taking care in the interpretation of data and taking into account everything that happened in the factory.

POSTER

SUGARCANE FACTORY TRIALS WITH DEXTRANASE ENZYME (from *Chaetomium gracile*)

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ABSTRACT

Dextran levels in sugarcane factories become high when cane shipments are delayed by bad weather, or when cane is frozen and thawed. Trials on addition of dextranase enzyme (made from *Chaetomium gracile*) at two sugarcane factories, when high levels of dextran were reported, are described. In one factory enzyme was added to the juice before lime addition. In the other factory enzyme was added at the evaporator station. Effects were measured across crystallization and in sugar. Effectiveness of enzyme in syrups traveling through pan boiling is discussed.

INTRODUCTION

Sugarcane, in field, transport and factory, is easily subject to microbial infection, especially by *Leuconostoc mesenteroides*, and to production of dextrans by these microorganisms. Delays between harvest and grinding, damage to cane by freezing and subsequent shipping delays, allow more time for infection and deterioration and can cause buildup of microorganisms and increase in dextran production. The microorganism that produces dextran, *Leuconostoc mesenteroides*, is particularly likely to build up in numbers on wet, muddy cane, and on cane with a lot of exposed tissue surface, from billet harvesting, or damage by machine, or injury from freeze, disease or pests (1,2,3).

Dextran levels in cane are usually controlled by good planning of cane deliveries, and good hygiene in the cane yard, the mills and throughout the factory. However, there are times when weather problems (storms, freeze) cause unavoidable damage to cane and delayed deliveries. In these cases, infection and dextran levels build up in cane before it reaches the factory. Experience, in Australia for the most part, has shown that dextranase enzyme can be added to factory process to break down the large dextran molecules into small pieces, reduce the negative effects of dextran on production, and reduce levels of dextran detectable (by haze test) in raw sugar (3,4,5,6).

In the 1996-97 season in Louisiana, two notable periods of bad weather occurred that affected cane condition. In late October, severe rain and hail storms stopped harvest and delivery, caused broken, lodged and damaged cane, and resulted in delayed deliveries of damaged cane. The second period occurred with a freeze in late December, when cane, standing or harvested, was frozen in the field. The freeze was quickly followed by warm weather which enhanced cane deterioration and dextran formation

in infected cane. Trials with dextranase enzyme (Dextranex™ from Genencor Corp., South Bend, Indiana) were made on evaporator syrup, after the October weather problems, and on mixed juice after the December-January freeze and subsequent warm-up. Both trials were successful: levels of dextran (by the haze method) were lowered in process materials and in raw sugar. Results from both trials are reported herein.

PROBLEMS CAUSED BY DEXTRANS

The major problem from dextran is that its presence indicates loss of sucrose, in cane, in harvesting and transport, in the mill yard and in the factory. Dextran in juice, syrups and sugars can cause false pol because dextran polarizes about three times as much as sucrose, and gives a falsely high pol. Dextran in solution increases viscosity, lowers evaporation rates and reduces heat transfer. It slows boiling times, and slows purging in centrifugals through the viscosity increase. An estimate of increase in molasses purity, or loss of sucrose to molasses caused by the presence of dextran, is that, for every 300 ppm dextran in syrup there is a 1% increase in molasses purity (3).

The effect that dextran has on sugar crystal shape, to elongate the crystal or cause “needle grain”, also increases loss to molasses by blinding centrifugal screens with elongated crystals (2). Most of these problems are more severe with high molecular weight dextrans (>500,000 Daltons); dextrans observed in sugarcane have an average molecular weight that is high. The chemical structure is shown in Figure 1 (7).

DEXTRANASE ENZYME

Dextranase enzyme, which can be made by several microorganisms and isolated for commercial use, can be added to sugarcane juice or syrup, at moderate temperatures and pH 5 - 8, to break dextran down into small (low molecular weight) units. Use of the enzyme does not remove the dextran, but it removes the problems associated with high molecular weight dextran, that is, increased viscosity, false pol increase, and high haze analysis results.

Microorganisms that can produce dextranase enzyme include *Penicillium* sp., *Aspergillus* sp., *Fusarium* sp., *Paecilomyces* sp., *Lipomyces* sp. and *Chaetomium gracile*. A dextranase from a *Penicillium* species is in commercial production (as may be others) and is in use outside the United States, but it is not approved for food manufacture in the United States. The dextranase made by *Chaetomium gracile* was developed in Japan, by Dr. Atsushi Hattori, of Sankyo Co. This enzyme (herein called dextranase-CG) has been used in Australia. A GRAS (generally recognized as safe) affirmation petition was filed with the U.S. Food and Drug Administration in 1995. It was accepted for filing, and assigned the number GRP 6G0420.

Reasons for the introduction and use of this new dextranase include:

1. Safety

2. Great range of pH and heat stability
3. Effectiveness in sugar processing.

DEXTRANASE-CG ADDITION TO CANE JUICE

These trials were conducted in early January, 1997, after a severe freeze in late December. Cane was very difficult to process. Dextranase enzyme was added to mixed juice, before liming or heating, at a rate of 6 g per ton of juice over a two day period. Cane entering the mill had been frozen and then thawed, when ambient temperatures increased so that much infection and deterioration were present in the badly wounded cane. Residence time for enzyme in juice, before lime was added and the juice heated, was about 12-15 minutes. Samples were taken before and after Dextranase-CG addition at hourly intervals. Typical before and after values are shown in Figure 2, as mg/kg (ppm) on a solids basis. The equation to "convert" haze readings, in mau (milliabsorbance units) from a spectrophotometer to mg/kg dextran, based on high molecular weight dextran, similar to naturally occurring dextran, is:

$$ppm = \frac{mau + 118}{0.66}$$

Results of tests, by haze analysis (8) showed a short treatment by dextranase reduced dextran levels by 50% to 85%. Before addition of the enzyme, the factory experienced severe difficulties in pan boiling, with low yields and high purity molasses. After use of Dextranase-CG, pan viscosities and molasses purities dropped back to normal for the end of crop. After use of Dextranase-CG, dextran tests in raw sugar produced showed a drop from over 2000 mau (before enzyme usage) to 300-400 mau (after enzyme usage) by haze tests.

DEXTRANASE ADDITION TO EVAPORATOR

In trials in October, 1996, after severe rain, wind and storms had caused cane breakage and lodging, and disturbed planned delivery systems, dextran levels were found to be high in incoming cane.

Dextranase-CG enzyme was added to the 4th (and last) effect of the main evaporator over a period of 8 hours. Samples were taken at 2, 4, 6 and 8 hours, after enzyme addition was begun. Samples of 3rd effect syrup (before enzyme addition), evaporator syrup, A-molasses and C-molasses were taken, and analyzed for dextran content by the haze test. Results are shown in Table 1.

The levels of dextran in syrup were reduced by 70-75% and in A molasses by 20% to 60%. Levels of dextran in raw sugar fell from 1500 mau on the previous day to 400 on the day of treatment with Dextranase-CG enzyme.

CONCLUSIONS

Dextranase-CG (informal name for dextranase from *Chaetomium gracile*), was stable and effective in sugarcane juice and syrup up to 85°C and 65 Brix. Except for the possibility of some activity loss at the point of application, (concentration problem), the enzyme appeared to have remained active throughout the process.

Enzyme Dextranase-CG, when added to cane juice before liming, with a short retention time of 10-12 minutes, was able to reduce dextran levels in juice by 50% to 85%, and in sugar produced from that juice by 90%. All dextran was determined by the haze test. The effect of enzyme Dextranase-CG, when added to the fourth evaporator effect, was to reduce dextran levels in syrup by up to 78%, and to continue to reduce dextran levels in A and C molasses by an average 50% to 60%.

The dextranase enzyme (from *Chaetomium gracile*) was shown to be effective at decreasing levels of dextran in juice, syrups and sugars from damaged cane.

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8. Dextran in raw sugar by a modified alcohol haze method. IGSI-15. ICUMSA Methods Book, 1994, Colney, Norwich, U.K.

Table 1. Decrease in dextran levels after syrup treatment by enzyme Dextranase-CG, averages over 18 hr.

	after addition: 4hrs	6hrs	8hrs
Evaporator syrup	78%	75%	-
A molasses	20%	40%	60%
C molasses	-	70%	60%

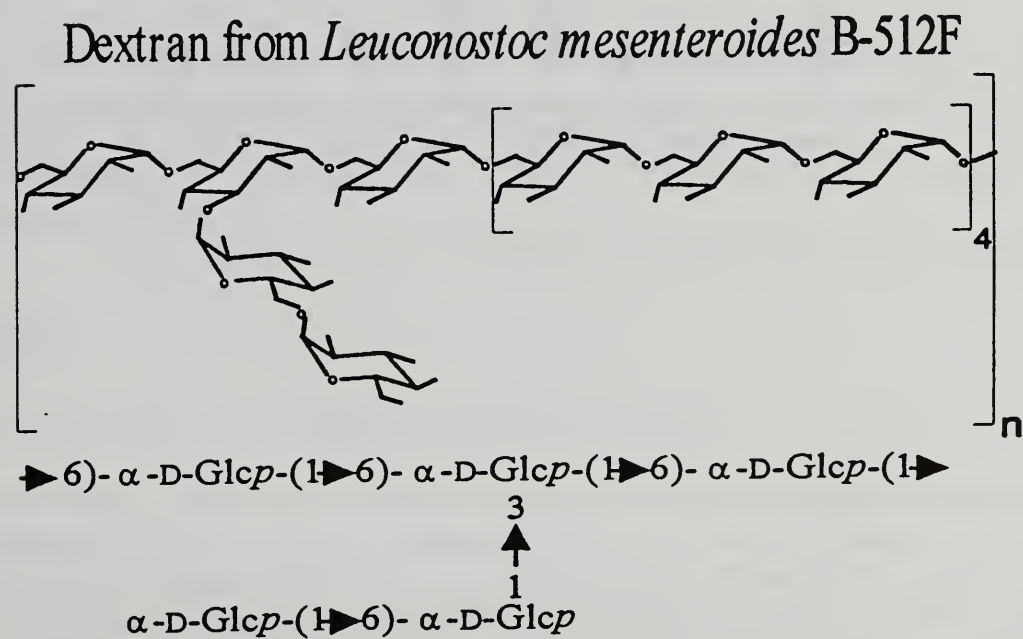


Figure 1. Structure of dextran.

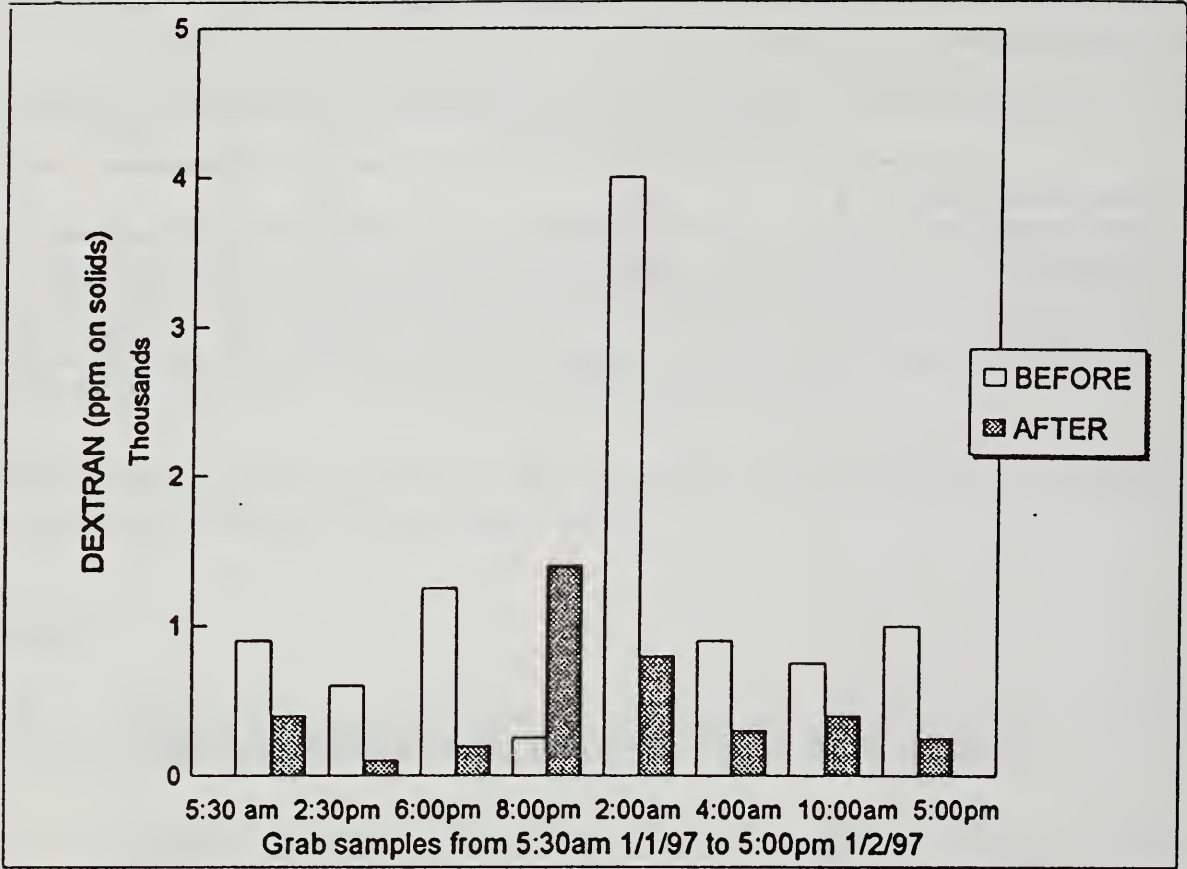


Figure 2. The effect of dextranase treatment on dextran in sugarcane juice.

POSTER

THE FATE OF SOLUBLE SUGARCANE POLYSACCHARIDES IN SUGAR MANUFACTURE

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¹Sugar Research Institute, Australia

²Sugar Processing Research Institute, Inc., New Orleans, Louisiana, USA

ABSTRACT

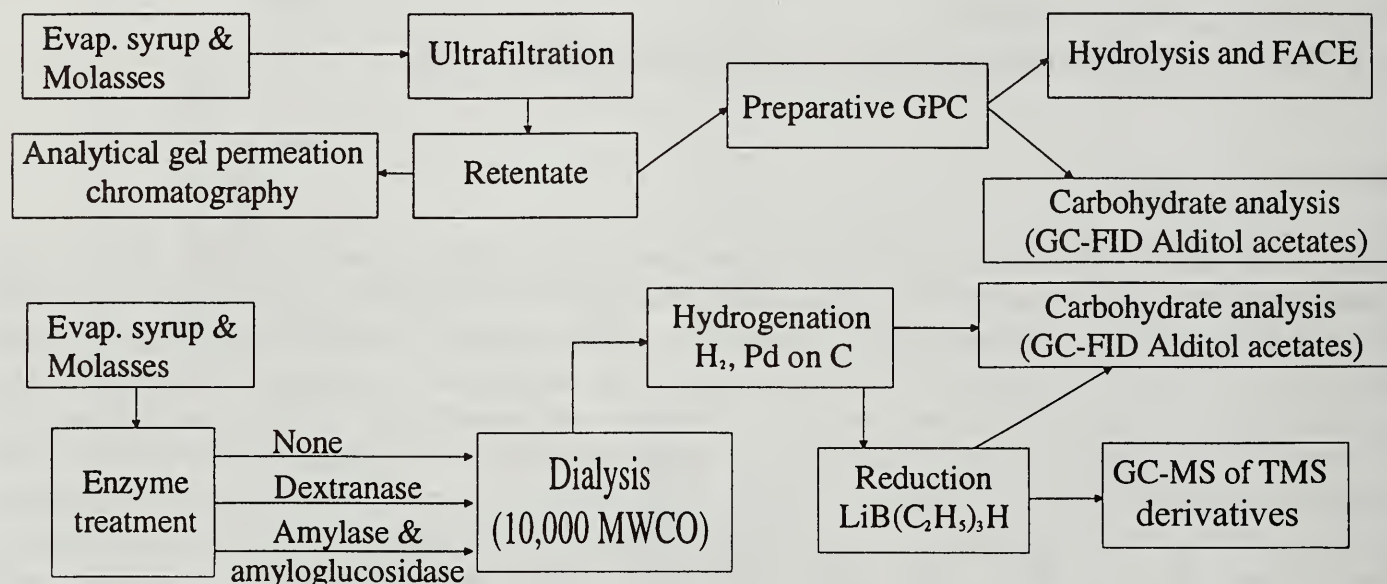
Milling of sugarcane (*Saccharum officinarum*) produces a juice which contains sucrose, other soluble material and particulates in suspension. After clarification and concentration by evaporation the resulting syrup contains most of the original soluble material (*viz.*, polysaccharides and other organic and inorganic compounds). The polysaccharides in juice, syrup and sugar are a mixture of extracted plant polysaccharides and microbial polysaccharides. Among them are starch and hemicellulose indigenous to the cane plant and dextran and possibly other polysaccharides derived from microbial infection of either the cane plant during harvest or material during processing. These polysaccharides have deleterious effects on raw sugar manufacture and some of them persist in the raw sugar product causing problems in further refining. Evaporator syrups and molasses samples collected during the 1995 Louisiana cane sugar campaign have been fractionated by several methods and the high molecular weight materials have been studied by analytical GPC and other techniques. The fate of these soluble polysaccharides in raw sugar manufacture is described and the implications to new technologies for sugar manufacture based on membrane filtration are discussed.

INTRODUCTION**POLYSACCHARIDES IN CANE SUGAR MANUFACTURE**

The juice extracted from sugarcane by milling contains a mixture of indigenous plant polysaccharides and microbial polysaccharides at a concentration of about 1 to 5% of the soluble solids. The juice also contains high molecular weight (HMW) colorant (>12,000 Da). These high molecular weight, soluble compounds are described in more detail in Table 1. Although they are only minor components of the juice, they have a detrimental effect on the process of raw sugar manufacture and persist in the raw sugar product at about 0.1 to 0.2% of the raw sugar.

EXPERIMENTAL - FLOW CHARTS

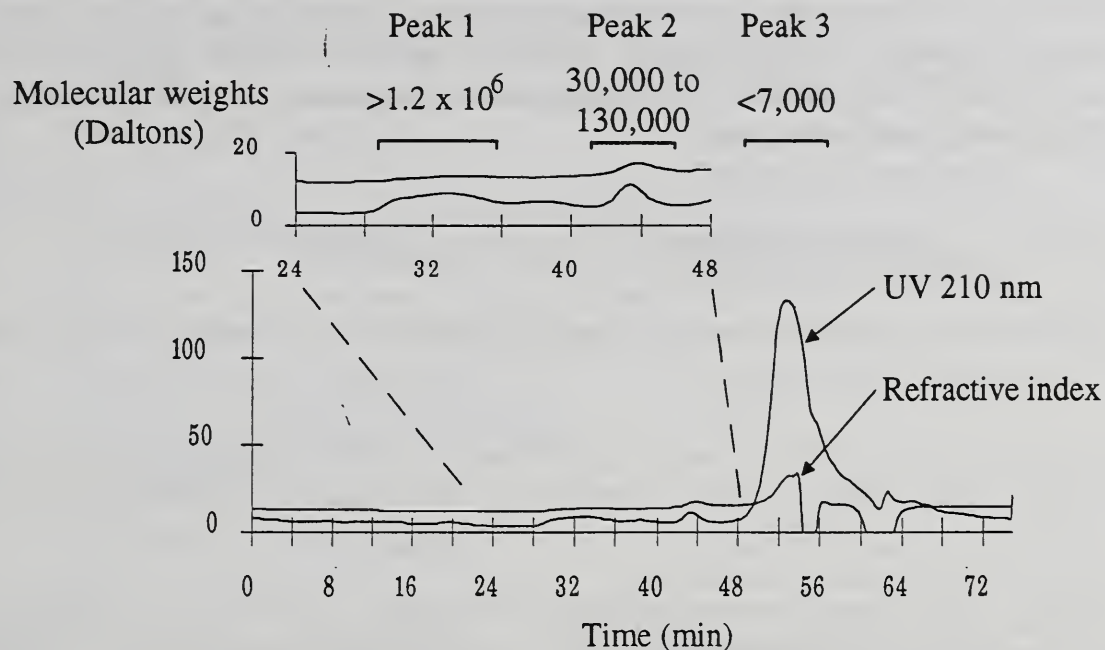
Evaporator syrups (clarified juice concentrated to *ca.* 60% soluble solids) and final molasses were collected from a Louisiana sugarcane mill during the 1995 harvest and analyzed according to the flow charts below.



GEL PERMEATION CHROMATOGRAPHY OF PROCESS STREAMS

Ultrafiltration retentates from evaporator syrup and final molasses when analyzed by GPC (PWXL Guard, G6000PWXL, G5000PWXL, G4000PWXL [Supelco], 0.6 mL/min 0.2M NaCl in 10% acetonitrile, R.I. & UV 210 nm detection) can be separated into 3 fractions. The molecular weights and average integration results for peaks 1 and 2 are shown in Table 2. Peaks 1 (as two fractions 1A & 1B) and 2 were collected and analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE) after acid hydrolysis; integration of the FACE bands are shown in Table 3 (these results were later confirmed by compositional analysis as alditol acetates).

GPC of process stream (final molasses).



FACE analysis of GPC fractions.



CROSS-LINKING OF POLYSACCHARIDES BY "POLYPHENOLIC BRIDGES"

The average integration values of GPC peaks 1 and 2 for several evaporator syrups and molasses samples (see Table 2) indicate that during processing the compounds in peak 1 are formed while compounds in peak 2 are consumed; both peaks contain polysaccharides (see Table 3). We speculate that peak 1 is a network of polysaccharides cross-linked by either ether or ester linkages with polyphenolic bridges. These 'phenolic' compounds are described in Table 1. Enzyme treatments prior to dialysis were used to disrupt the polysaccharide part of the network. Hydrogenation followed by reduction of the dialyzate was used to disrupt the polyphenolic part of the network, thereby establishing the presence of bridges.

The detailed results of compositional analysis are not reported here; in general, any treatment (enzymic or chemical) that disrupted the network increased the proportion of arabinose and xylose. The Ara/Xyl ratio remained relatively constant. GC-MS analysis of the low molecular weight products of hydrogenation and reduction confirmed the presence of lignophenolic monomers, including coniferyl and other methoxyphenylglycerols and quinic acid).

CONCLUSIONS - FATE OF SOLUBLE POLYSACCHARIDES

The juice extracted from sugarcane contains discrete polysaccharides, some with ester-linked phenolic groups.

Cross-linking and the formation of "phenolic bridges" are shown by the changes in GPC peak areas from evaporator syrup to final molasses, and by analysis of the products of hydrogenation and reductive cleavage.

During processing, cross-linking of polysaccharides is initiated by heat and oxidative free radical reactions and forms larger HMW compounds, with intense color and high viscosity. These cross-linked compounds are the source of process problems.

New process technologies (based on ultrafiltration) remove lignocellulosic and soluble polysaccharides and prevent the formation of the polysaccharide polyphenolic network. Consequently, the process streams are less colored and less viscous, and more sugar of higher quality can be produced.

Table 1. Source of polymers and their effects on cane sugar manufacture.

Compound	Source	Effect on sucrose manufacture
Soluble plant polysaccharides	Indigenous to cane plant	- Lowers yield and quality of products - Causes acid beverage floc
Starch	Indigenous to cane plant	- Increases viscosity - Lowers yield of products
HMW colorant (>12,000 Da)	Degradation and condensation of sugars and amino acids; indigenous polyphenolic pigments and flavonoid moieties; soluble lignin-carbohydrate complexes	- Lowers yield and quality of product - Can cause sugar products to discolor in storage
Dextran	Soil/airborne microorganisms	- Increases viscosity and lowers yield - Distorts growth of sugar crystals
Other microbial polysaccharides	Soil/airborne microorganisms	- Lowers yield of products

Table 2. GPC average peak areas.

Process stream	GPC peak areas	
	1	2
Evaporator syrup	64	855
Final molasses	369	188

Table 3. FACE analysis.

Band identity	Relative band intensity		
	Peak 1A	Peak 1B	Peak 2
Arabinose	28.6	39.4	56.0
Xylose	8.4	12.7	5.0
Glucose	46.0	28.6	1.4
Galactose	17.0	19.3	37.6

POSTER

CLARIFICATION OF MIXED JUICE FROM FRESH AND STALE SUGARCANE: INVESTIGATION OF SUGAR LOSSES

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ABSTRACT

Changes in levels of sucrose, glucose and fructose, across a sugarcane factory's clarification process were investigated. The cooperating factory uses a "cold liming" process, where lime is added to the mixed juice before heating, prior to clarification. Samples of mixed, limed, heated limed and clarified juice were obtained hourly over a seven hour sampling period. This sampling period was repeated seven times across the grinding season; in the last two sampling periods the crushed cane was stale because the field cane had been subjected to freezing, followed by warm weather conditions. Ion chromatography with integrated pulsed amperometric detection (IC-IPAD), an accurate sugar analysis technique, was used to measure directly sucrose, glucose and fructose levels.

For the fresh cane, color, turbidity and pH of the mixed juice improved across the season because of increasing cane maturity. Generally, color formed in the lime tank because of the alkaline degradation of glucose and fructose (invert). Minimum sucrose loss across the clarification process was 3.39%. Very high levels of glucose and fructose (both often >7% on a °Brix basis) occurred in the mixed juice from the stale cane, as well as high levels of dextran. Glucose/fructose ratios were also higher in stale cane, because of more associated trash, which gave falsely high polys and purities, especially in the clarified juice. Turbidity removal in the clarification tanks was worse with stale cane. The stale cane was very acidic and pH control of its mixed juice in the lime tanks was exceptionally difficult, causing acid degradation of sucrose (inversion) to occur in the tanks. pH control in the lime tanks was erratic across the grinding season and it is, therefore, recommended that lime should be added as lime saccharate in the future. A full statistical analysis of the data is presented and discussed.

INTRODUCTION

The primary aim of clarification in raw sugar manufacture is to remove from the mixed juice (**MJ**) the maximum quantity of impurities, more specifically particles, at the earliest stage. The classical simple defecation process is "cold liming", where the cold **MJ** is limed and rapidly heated to final temperature before settling in a clarification tank. The advantages of "cold liming" over "hot liming" are considered to be less inversion and sugar destruction, whereas the major disadvantage is the need to add high levels of lime. In general, sufficient lime is added to neutralize the organic acids present in the **MJ**, after which the temperature is raised to 200 °F or more. This lime and heat treatment forms a heavy precipitate of complex

composition (primarily calcium phosphate (1), but also includes colloidal precipitation), part lighter and part heavier than the juice, the contents of which contain insoluble lime salts. The separation or settling of the precipitate, aided by flocculating polymers, occurs in the clarification tank. The degree of clarification has a great impact on the boiling house operations, the sugar yield and refining quality of the raw sugar.

It is generally recommended that liming the MJ to pH 8.5 or above should be avoided, otherwise excess lime salts, and alkaline degradation of invert (glucose and fructose) with increased color and viscosity may occur. Retention time in the lime tanks also has a critical effect on the process. Prolonged (usually up to 15 min) and rigorous stirring after liming permits improved floc formation, a better settling rate in the clarification tank, increased rise in gravity purity, lower turbidity, less increase in residual calcium, and a lower final mud volume. Furthermore, the increase in calcium salts is less than when no or poor stirring is employed. Other evidence (2) indicates that the phosphate content of the juice is also an important factor in efficient clarification and, therefore, juices with adequate amounts of inorganic free phosphate (>300 ppm w/v) are most desirable.

Clarification of raw juice from poor quality, "stale" or deteriorated cane that has been exposed to freezing, followed by warm weather conditions, still poses a serious technological problem, which has yet to be solved. (Note: "stale" cane used here, refers to freeze deteriorated cane that was not left in the field or factory yard for a prolonged time). The present opinion is that filtration and sedimentation problems during clarification of such stale cane are totally related to the harmful action of dextran. However, the adverse effects of invert formation and products formed from invert degradation should not be underestimated. In this study, such extreme environmental conditions occurred at the end of the cane grinding season, allowing us to investigate the impact of high invert levels and their destruction, on the efficiency of the clarification process. The use of the direct and accurate technique of ion chromatography with integrated pulsed amperometric detection (IC-IPAD) allowed for a more accurate determination of sucrose and invert levels across the clarification process. This study was, therefore, undertaken to determine the efficiency of the "cold lime" clarification process using fresh and stale sugarcane. It is also part of a larger, ongoing study to determine sugar degradation and losses across different sugar industry unit processes.

EXPERIMENTAL

This investigation of sugar loss across "cold lime" clarification was performed at a Louisiana sugar cane factory during the 1996/97 grinding season. Average factory flow throughput for the grinding season was ~120,000 gallons/h and approximately 30% of the cane ground was billeted cane (personal communication, factory personnel). The average residence time across the lime clarification unit process was approximately 1 h 55 min, and a flow diagram of the process is shown in Figure 1. Lime was mixed with water and added continuously to the lime tank as a slurry. The lime tanks were operated at ambient temperature with a target pH of approximately 7.5 for the limed juice. The limed juice was then heated to 225 °F in a flash heater. The target temperature in the clarifier tank was 200 °F and the target pH of the clarified juice was approximately 6.7. Three flocculants (StockhausenTM, TaloSepTM and CartofloxTM) were added to the clarification tank and mud filters at 5 ppm on clarified juice. No phosphate was added to the cane juice.

Sampling

Unscreened mixed juice (**MJ**; also known as dilute juice) from the mill end of the factory, limed juice (**LJ**), heated limed juice (**HLJ**) and clarified juice (**CJ**) were obtained hourly over a seven hour period. There was a 15 min delay between sampling **MJ** and **LJ**, a 2.5 min delay between **LJ** and **HLJ**, and a further 1 h 37 min delay between sampling **HLJ** and **CJ** (residence time in the clarifier tank was calculated using the tank dimensions and average flow rate). The seven hour sampling period was repeated seven times across the grinding season, in order to cover sugarcane, environmental and process parameter variations. Figure 2 illustrates when sampling periods occurred as well as local weather conditions.

At the end of the campaign, the field sugarcane was exposed to freezing followed by unusually warm weather conditions, which are illustrated in Fig. 2, causing a marked deterioration in cane quality and a dramatic increase in dextran. The policy of the mill was to add dextranase enzyme to reduce dextran levels. Dextranase was added just before lime clarification in a tank, which had previously been used as one of two factory lime tanks, and reduced the high dextran levels to 10% of its original level (3). As one of the lime tanks was no longer in use the residence time was halved from 15 to 7 ½ mins. Sampling periods 6 and 7 occurred under such extreme conditions.

All samples were immediately quenched and stored in ice to prevent further decomposition, until they were transported to and stored in a -43°C freezer.

Pol

26.00 g/100.00 mL samples were mixed with Filtercel™ and filtered through Whatman 91 paper (4). Clarified samples were measured in duplicate at 589 and 880 nm in a 20 cm cell, on a Rudolph Autopol 880 Polarimeter calibrated in ISS (Z scale) at 20 °C.

°Brix

Sample °Brix was measured at least in triplicate, using a Leica Abbe Mark II Refractometer with a crosshair reticule.

pH

The pH of samples was measured at room temperature (~23 °C), using an Ingold™ combination pH electrode calibrated at room temperature using two different pH buffers. The electrode was connected to a Metrohm 716 DMS pH meter.

Color and Turbidity

Samples were diluted in triethanolamine/hydrochloric acid buffer (pH 7) and filtered through Filtercel™. Color and turbidity were measured at absorbance 420 nm and calculated according to the official ICUMSA method GS2/3-9 (1994).

Total Polysaccharides

Polysaccharides were precipitated with absolute ethanol and total polysaccharides were determined using the method of Roberts (5)

Sucrose, Glucose and Fructose

Diluted samples were filtered through a 0.45µm filter. Sucrose, glucose and fructose were determined by Ion Chromatography (IC) using a Dionex (Sunnyvale, CA, USA) BioLC instrument equipped with a quaternary gradient pump module (GPM). Carbohydrates were separated on Dionex CarboPac PA guard (25 x 4 mm) and PA-1 analytical (250 x 4 mm) anion exchange columns, at a flow rate of 1.0 mL/min at ambient temperature (~25 °C). Column eluent conditions were: 16 mM NaOH isocratic (inject; 0.0-2.0 min), a gradient of 16-160 mM NaOH (2.0-35.0 min), followed by isocratic 200 mM NaOH (35.0-40.0 min), and return to 16 mM NaOH (40.0-45.0 min) to re-equilibrate the column with the initial mobile phase prior to the next sample injection. Carbohydrates (25 µL) were detected using integrated pulsed amperometric detection (IPAD). The detector was equipped with Au working and Ag/AgCl reference electrodes, operating with the following working electrode pulse potentials and durations: $E_1=+0.05$ V ($t_0=0.00$ s), $E_2=0.05$ V ($t_1=0.42$ s), $E_3=+0.75$ V ($t_3=0.43$ s), $E_4=+0.75$ V ($t_4=0.60$ s), $E_5=-0.15$ V ($t_5=0.61$ s), $E_6=-0.15$ V ($t_6=0.96$ s). The duration of the IPAD integration interval was set at 0.2-0.4 s. Using a Spectra-Physics SP8880 autoinjector and Dionex peaknet chromatography software, runs were accumulated of multiple samples and standards. The standards were myo-inositol, glucosamine-HCl (internal standard), glucose, fructose, sucrose, raffinose and stachyose. Seven different levels of the standards were run first, and standard curves were generated (glucose and fructose ranged from 1 to 7 ppm, sucrose 1 to 13 ppm) to test linearity in multiple runs and generate area response factors. Weight diluted samples were run in duplicate. Glucose and fructose were quantified from different chromatographic runs from sucrose, because sucrose occurred at much higher concentrations in the samples. Response factors were generated for each of the carbohydrates using internal standard calibrations and check standards.

Statistical Analysis

Data were analyzed using PC-SAS 6.12 (SAS Institute, NC) software. Factors analyzed included sample type, sampling period, and hour of collection within the sampling period. As a preliminary Analysis of Variance indicated that there was no statistical difference between collection hours, they were consequently removed as a factor in all subsequent analyses, which increased the error degrees of freedom. PROC GLM

was used to test single factor models, and means were tested using either a T-test or Duncan's New Multiple Range Test.

RESULTS

Efficiency of the Clarification Process: Turbidity Removal

Average turbidity values of the mixed juice (MJ) decreased ($P < .089$) across the grinding season up to sampling period 5, as shown in Figure 3a, and this reflects the improved and more mature cane, and better harvesting practices. The immature cane, ripened with PoladoTM (a chemical ripener) at the beginning of the harvest season, had more trash associated with it as the cane was shorter and harvested with tops, which contributed to the high turbidity levels. Generally, turbidity increased in the limed samples because of the added lime particles, then decreased in the heated juice with the onset of settling; as expected, turbidity decreased markedly in the clarification tanks. The anomalous increase in turbidity in the CJ from sampling period 2 is attributed to the increase in total polysaccharides (see Figure 4) in the clarification tanks, which is most likely a result of contamination problems and highlights the need for good factory sanitation. The turbidity increase in the lime tank in sampling periods 6 and 7 reflects the extra amounts of lime which had to be added to the stale, acidic cane. Samples from periods 6 and 7 were visually more turbid and difficult to filter; furthermore, it can be seen in Figure 3a that their measured turbidity values were very high. Similarly, in Hawaii, juices may average 2-4% insoluble solids, and in wet weather insoluble solids often increase to 6-7% (2). Average % turbidity decreases across the clarification process are shown in Figure 3b. The worst turbidity removal occurred in the stale cane samples from sampling period 6, although there was a slight improvement in period 7. There was no overall reduction in turbidity in the period 2 samples, because of the stated contamination problems.

Process pH Levels

Average pH levels of MJ, LJ, HLJ and CJ, from each sampling period, are shown in Figure 5. The average MJ pH increased slightly, but significantly ($P < .0001$), up to sampling period 5, reflecting the increased maturity and better quality cane. The addition of slurried lime increases the juice pH, however, as shown in Figure 5, the pH of the LJ samples varied considerably (pH range 4.6 to 9.7) across the grinding season as well as across collection hours. This strongly suggests that pH control in the lime tanks was somewhat erratic, and requires further improvement to reduce unwanted sugar degradation reactions. Moreover, the pH of the LJ in period 1 was high, i.e. >8.5 , which are strong conditions for the alkaline degradation of invert sugars to occur, with unwanted formation of color and polymer compounds, and organic acids. Up to sampling period 5, the pH of the HLJ samples generally decreased, indicating the lime had been already completely solubilized in the lime tanks before heating. Furthermore, this drop in pH on heating could be a function of the amount of phosphate in the juice; the higher the phosphate content the more marked the difference in pH before and after heating (2). Lime reacts with the phosphate to form several basic calcium phosphates; the more basic calcium phosphates are more insoluble and precipitate in the mud, which leaves the solution more acidic. Furthermore, optimum phosphate concentrations in cane are known to increase with the age of cane (1) and vary with geographical location and soil type.

Levels of pH for all the different samples in periods 6 and 7 were dramatically different. **MJ** levels were very low (lowest value pH 4.7) which highlights how acidic the stale cane was. Although extra lime had to be added to neutralize the acidic **MJ**, the pH of the **LJ** did not significantly increase, which left the **LJ** pH at dangerously low levels for acid degradation of sucrose (inversion) to occur (see section on Invert Levels). As only one lime tank was in use (see Experimental section) the retention time of **LJ** in the lime tank had been halved; however, this does not entirely explain the low pH levels, which must also be due to poor mixing. Lime was only fully solubilized when heated which caused the **HLJ** pHs to increase, and the pH of the **CJ** samples was still relatively high compared to those in sampling periods 1 to 5, suggesting a higher degree of lime salts were still present in the clarified juice. This would have led to poor working, low grade materials in the boiling house and to dark, gummy raw sugars, as the amount of reversible colloids or gummy substances increases almost linearly with increase of lime salts in the **CJ** (2).

Color Formation

Average color values across the grinding season are shown in Figure 6, and it is clearly shown that color of the **MJ** decreased dramatically, by approximately 7,500 ICUMSA units, over the first 5 sampling periods. Furthermore, color values of all the samples were statistically ($P < .0001$) much higher in sampling periods 1 and 2. This strongly indicates further that the cane was improving with maturity and better harvesting practices. The increase in **MJ** color in sampling periods 6 and 7, reflects the composition of the more acidic, stale cane; acid degradation reactions of sucrose in the field cane and in subsequent cane crushing and grinding, caused color to form. Figure 7 shows the average color changes in the lime and clarification tanks. Except for the stale cane in sampling periods 6 and 7, where acid degradation conditions prevailed in the lime tanks, color formed in the lime tanks because of the alkaline degradation of invert. The variation in color changes reflects the variation and difficulty of pH control in the lime tanks. Figure 7 also illustrates that, except for sampling periods 1 and 2, color removal was greater than color formation in the clarification tanks. The color formation in periods 1 and 2, most likely reflects the higher amounts of cane tops associated with the PoladoTM ripened immature cane at the beginning of the season.

Invert Levels

Invert (glucose and fructose) levels can be used to diagnose if and what type of sugar degradation has occurred in the factory. An increase in glucose and fructose indicates that acid degradation (inversion) of sucrose has occurred. In comparison, a reduction in invert levels usually indicates that the alkaline degradation of invert has occurred.

Invert levels were carefully determined with a very accurate IC-IPAD quantitation method. Glucose and fructose were measured in two hourly samples, randomly chosen from each sampling period, and are illustrated in Figures 8 and 9, respectively. As expected, invert levels, quoted as a % on °Brix basis, were generally similar.

Up to sampling period 5, glucose and fructose values were <4% on a °Brix basis, with the notable exception of the anomalous samples from sampling period 2. Furthermore, up to sampling period 5, glucose and

fructose generally decreased in the **LJ** samples which confirms that significant alkaline degradation of these invert sugars occurred, which is responsible for the increase in color (see Figures 6 and 7). Under optimum conditions, as in period 3 where the coldest ambient temperatures occurred, alkaline degradation of invert should be kept to a minimum across the process to avoid excessive color and polymer formation. It can also be seen in Figures 8 and 9, there were numerous samples where there was an increase in glucose and fructose in the clarification tanks, because acid degradation of sucrose (inversion) had occurred.

There were dramatic increases in glucose and fructose levels in the **MJ** from the stale cane (periods 6 and 7) samples (both often >7% on a °Brix basis), and further amounts were also formed in the lime tank, even though the retention time of the **LJ** had been halved. This was because of the acid pH values of the **LJ** samples (see Figure 5) caused by poor mixing of lime, which allowed excessive acid degradation of sucrose to occur and the subsequent formation of glucose and fructose. This was also previously (6) confirmed by the formation of kestoses, products of the acid degradation of sucrose). These very high levels of glucose and fructose in the **LJ** were then markedly reduced on heating, due to the solubilization of lime causing alkaline degradation of glucose and fructose to occur and excessive color formation (see Figure 5). Due to the severe acid conditions of the stale cane, acid degradation of sucrose occurred again in the clarification tanks, adding even more glucose and fructose in the **CJ**. Consequently, the material entering the boiling house not only had high dextran levels (see Figure 4), but also high levels of glucose and fructose with color and polymers from subsequent degradation reactions of glucose and fructose. These results strongly suggest that, in the processing of stale cane, the control of glucose and fructose levels is as critical as the control of dextran levels. High levels of glucose and fructose in syrup will, of course, increase losses of sucrose to molasses.

Glucose/fructose (G/F) ratios were calculated and are shown in Figure 10. Except for the anomalous sampling period 2, up to sampling period 5 there were no significant changes in these ratios for all samples. However, there was a significant increase in the variation and value ($P < .028$) of the G/F ratios for the stale cane **HLJ** and **CJ** samples (periods 6 and 7). The higher G/F ratios are most likely indicative of the larger amounts of trash associated with the stale cane because, in trash, glucose occurs in greater concentration than fructose. However, there are often several sets of competing conditions that can affect G/F ratios in later factory process stages. In comparison to the cane and unheated mill and mixed juices, where the level of trash and degree of microbial infection predominate, for the juice in subsequent factory processes, sugar degradation reactions often participate as well. High levels of infection with *Leuconostoc mesenteroides* produce, from sucrose, dextran and excess amounts of fructose, which causes fructose levels to be greater than glucose levels (7). In general, at acid pH, glucose degrades more rapidly than fructose and vice versa in alkaline conditions.

Sucrose Levels

Sucrose levels across the clarification process were also determined using IC-IPAD and were measured in two hourly samples, randomly chosen from sampling periods 1, 3 and 6. Sucrose levels, quoted on a % °Brix basis, are listed in Table 1.

Sucrose occurs in higher concentrations in the clarification juices than glucose and fructose, and is more difficult to measure accurately. The absolute sucrose values in Table 1 are only approximations, although the relative changes in values across the clarification process are more accurate. Nevertheless, certain generalizations can be drawn from these results. Sucrose levels were obviously lower in the stale cane samples in period 6, and sucrose losses were greater too. The more acidic nature of the stale cane caused acid degradation of sucrose (inversion) to occur and the formation of very high levels of invert (see Figures 8 and 9). Generally, sucrose decreased in the lime tanks, especially in the stale cane **LJ** samples from period 6, where considerably more acidic inversion of sucrose occurred (see Figures 5, 8 and 9). However, the addition of lime would have contributed to the °Brix values, and as the sucrose levels are quoted on a °Brix basis, this may have contributed to the lower levels of sucrose in the lime tanks. Moreover, on settling of lime and other impurities in the **HLJ** and **CJ** samples, sucrose levels increased in certain samples (see Table 1), which further suggests °Brix values adversely affected the estimation of sucrose levels in the lime tank. Generally, marked sucrose losses occurred across the clarification process. Even under the more optimum clarification conditions in sampling period 3, where the coldest ambient temperatures occurred, there was still a total loss of 3.39%, with 1.3% of this loss occurring in the clarification tank.

Meaningful attempts to measure sucrose losses accurately across unit processes, especially the evaporation process, in sugar cane factories have been undertaken by various researchers (8, 9) using gas chromatography and IC-IPAD techniques, respectively. Because low levels of invert can be measured more accurately than the larger quantities of sucrose, sucrose losses were measured as increases in glucose to sucrose ratios. However, as data in this paper indicate, glucose is destroyed and formed across the clarification process, therefore, glucose to sucrose ratios would not be meaningful. The most accurate determination of sucrose loss across the clarification process would be to analyze for a stable degradation product, i.e., a marker compound. Possible oligosaccharide markers have been reported by Eggleston et al (6, 10).

°Brix, Pol and Purity Values

°Brix, pol and purity values are shown in Figure 11. There were no significant differences amongst the °Brix values for all samples up to period 5. However, °Brix was significantly ($P < .0001$) less in weeks 6 and 7 stale cane samples. This stale cane had visibly more trash associated with it, which absorbed more of the water initially used to clean the cane, causing the °Brix values to be lower.

Purity of the **MJ** generally increased across the grinding season up to period 5, further reflecting the improved and more mature cane, and better harvesting practices. The low purities of the stale cane (periods 6 and 7) are because of the larger amounts of associated trash. An increase in apparent purity between **MJ** and **CJ**, formerly considered a criterion of the efficiency of the clarification process, is now generally recognized as having little value and may actually be misleading (2). The rise could be due to destruction of fructose which causes a more positive effect on pol, or the formation of a sugar degradation product with a positive pol value. Purity values were generally higher than sucrose levels (see Table 1), further suggesting that they cannot be used to indicate sucrose levels and losses.

MAJOR CONCLUSIONS

Fresh Cane

Maturity of the cane at time of harvest had a dramatic effect on the clarification process: as the grinding season progressed and the cane became more mature, juice quality and clarification improved, although the extreme environmental conditions at the end of the season had an overriding effect. Cane juice at the beginning of the season had dramatically high color values because of the higher amounts of tops associated with the shorter, PoladoTM ripened cane. pH control was somewhat erratic across the grinding season and although improved stirring in the lime tank should improve control, the addition of lime as saccharate would give even more superior performance. Considerable alkaline degradation of invert occurred in the lime tanks, and minimum sucrose losses across the clarification process were calculated at 3.39%.

Freeze Deteriorated, Stale Cane - Effect of Extreme Environmental Conditions on Juice Quality

The stale cane had lower juice °Brix and sucrose values and markedly higher turbidity values. Turbidity removal on clarification was worse in stale cane. Stale cane not only had very high total polysaccharide (and dextran) levels, but very high invert levels too. Invert levels were further increased because of poor lime mixing in the lime tank, which caused inversion of sucrose to occur. On heating the lime dissolved and caused unwanted alkaline degradation of invert, with polymeric colorant and organic acid formation. Glucose/fructose ratios were higher, because of the higher amounts of associated trash and mud, causing falsely high polys in the clarified juice.

Recommendations for Improved Control of Cold Lime Clarification

1. Lime added as saccharate (lime dissolved in sucrose solution, such as an evaporator syrup, typically in a 1:7 ratio) would improve the pH control of the limed juice and handling of the lime.
2. Freeze deteriorated, stale cane requires increased stirring and/or higher retention times in the lime tanks, to avoid excessive inversion from occurring, even if more lime is added. More frequent and careful monitoring of pH is also recommended.
3. Shorter retention time in the clarifier tank is required.
4. Correct sanitization methods must be used to avoid contamination problems.

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Table 1. Changes in sucrose concentrations^a across the “cold lime” clarification process.

Sample	Average % sucrose values on a °Brix basis				
	Sampling period 1 3Hh ^b	Sampling period 1 5h ^b	Sampling period 3 0h ^b	Sampling period 6 2h ^b	Sampling period 6 5h ^b
Mixed juice	63.45	64.34	82.30	50.02	62.62
Limed juice	59.64	58.83	81.42	29.38	42.80
Heated limed juice	63.30	ND ^c	80.21	ND	56.94
Clarified juice	62.43	59.83	78.91	58.25	52.07

^a Determined using IC-IPAD - see Experimental Section

^b Sucrose levels were measured in randomly chosen hourly samples

^c ND = not determined

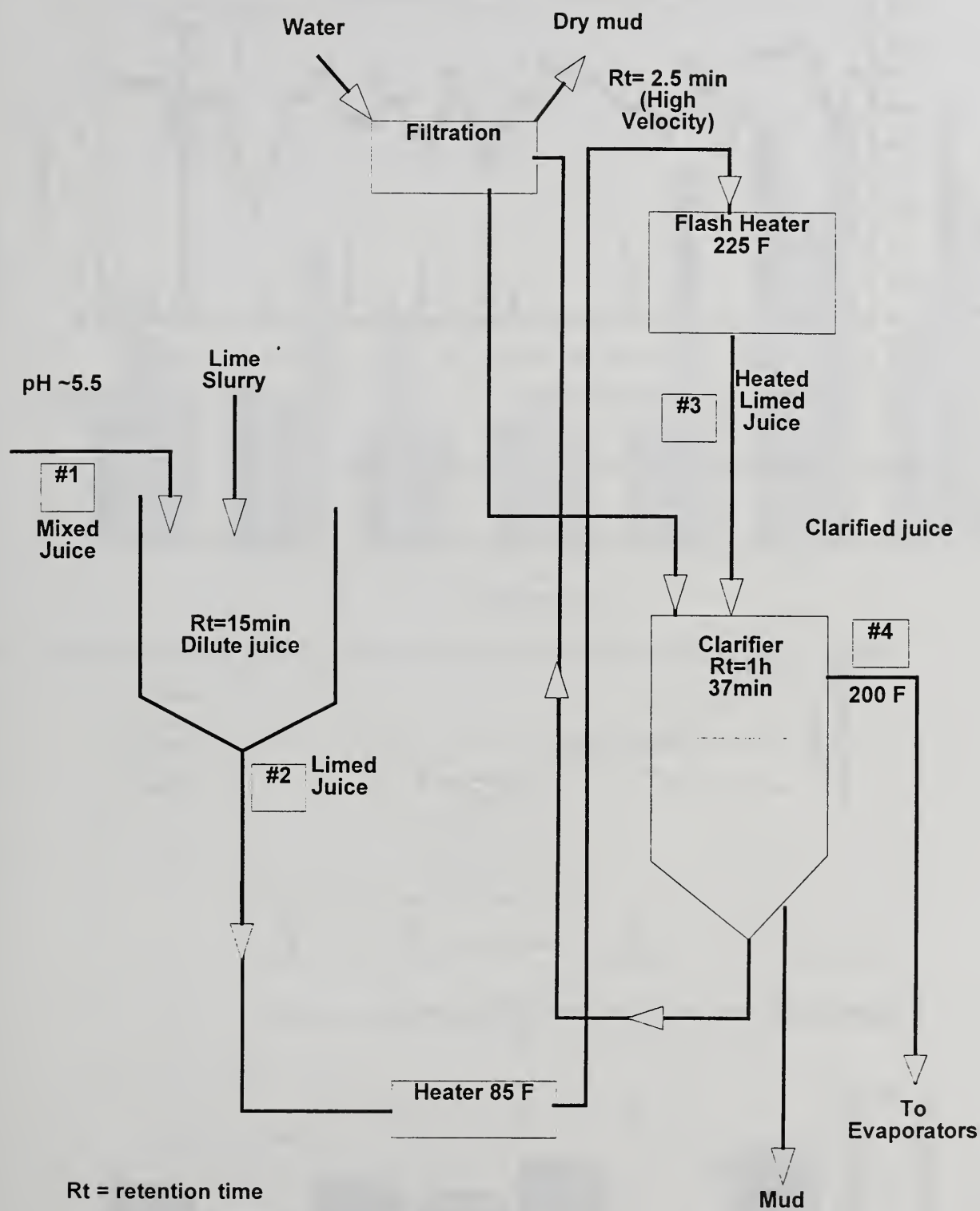


Figure 1. Flow diagram of "cold lime" clarification process.

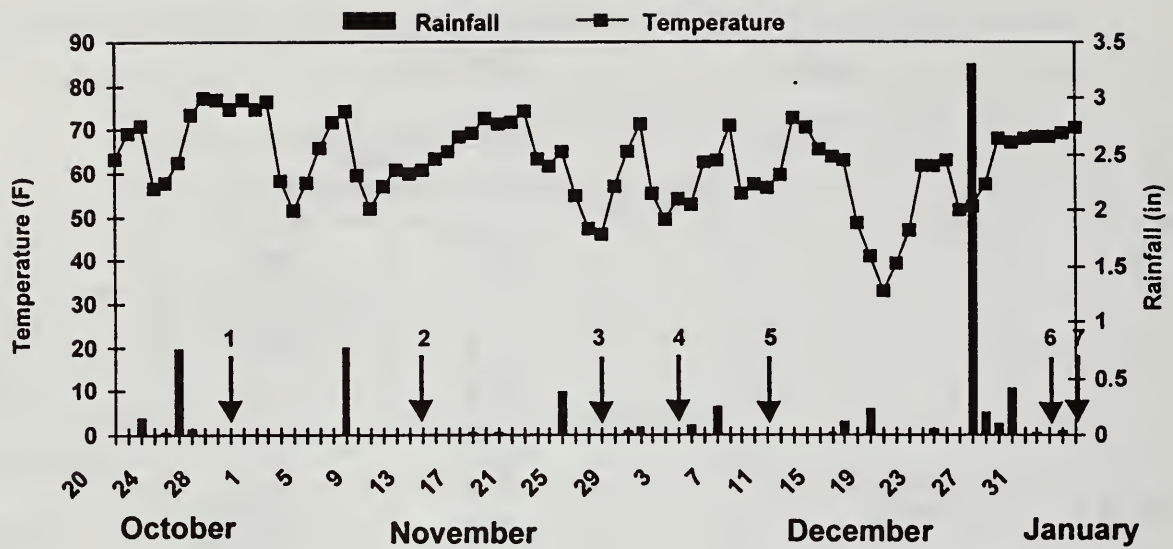


Figure 2. Mean rainfall and temperature data, with sampling dates.

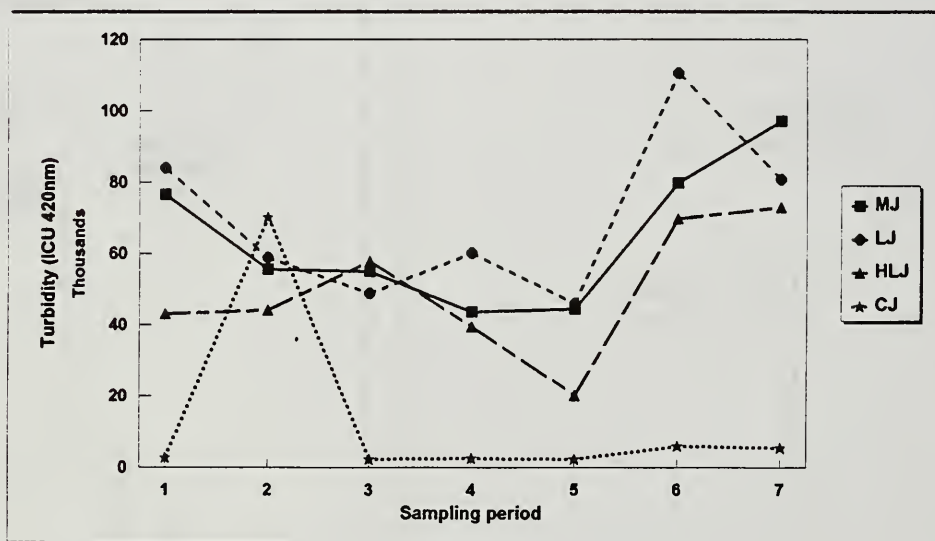


Figure 3a. Average turbidity values across the grinding season.

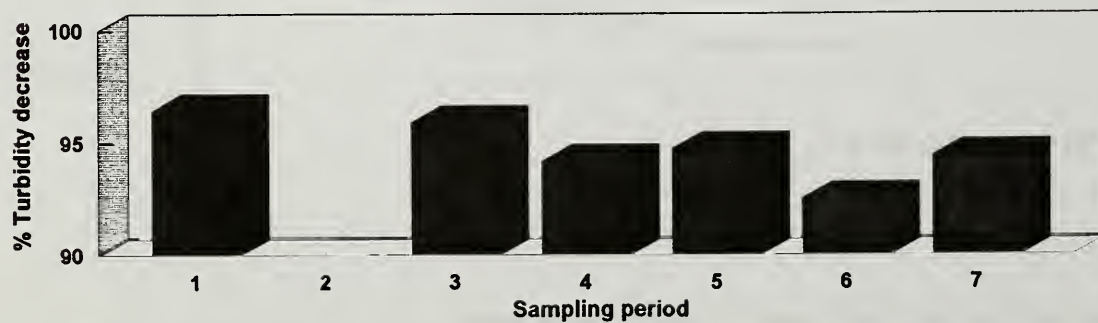


Figure 3b. % turbidity decrease across the clarification process.

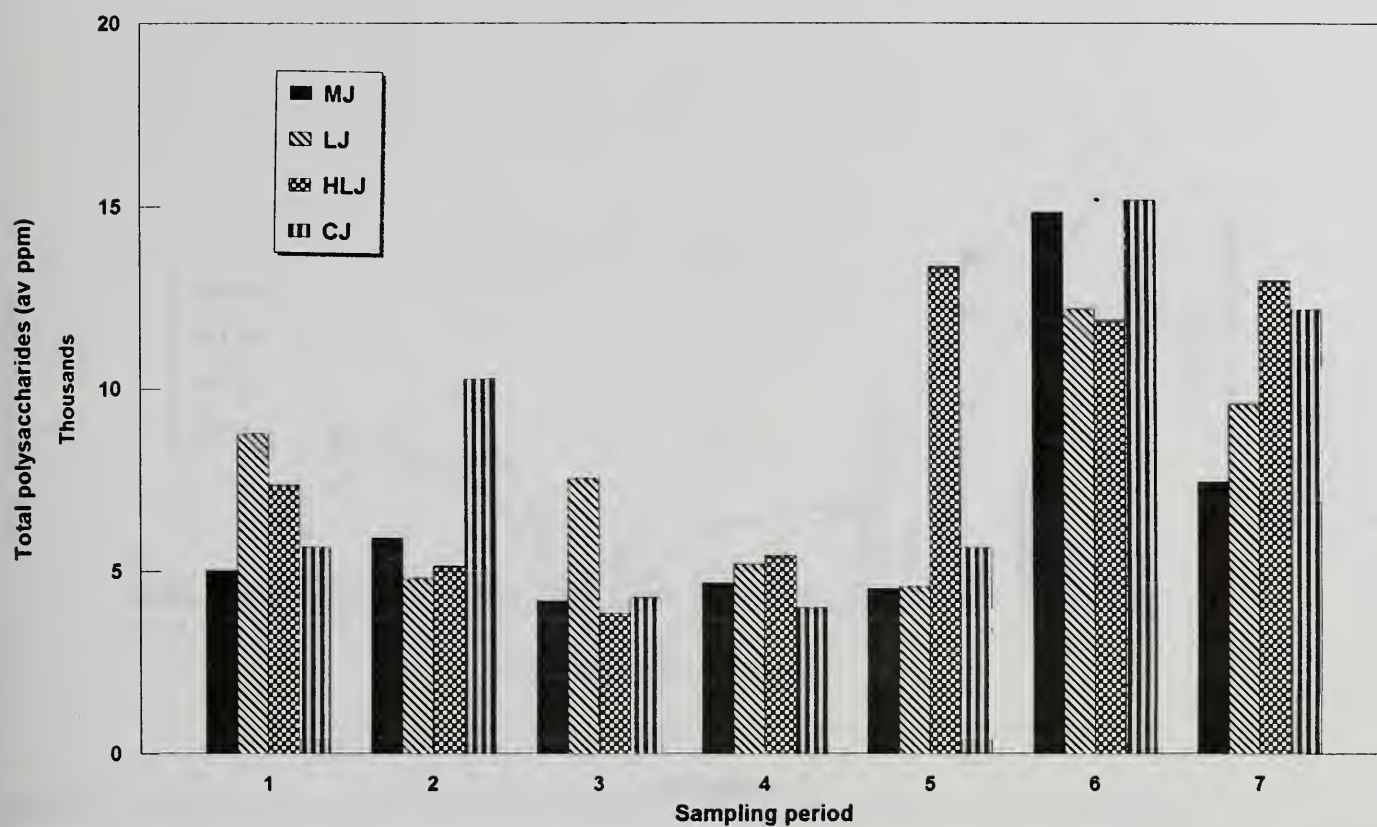


Figure 4. Total polysaccharides across the grinding season (one random hourly sample).

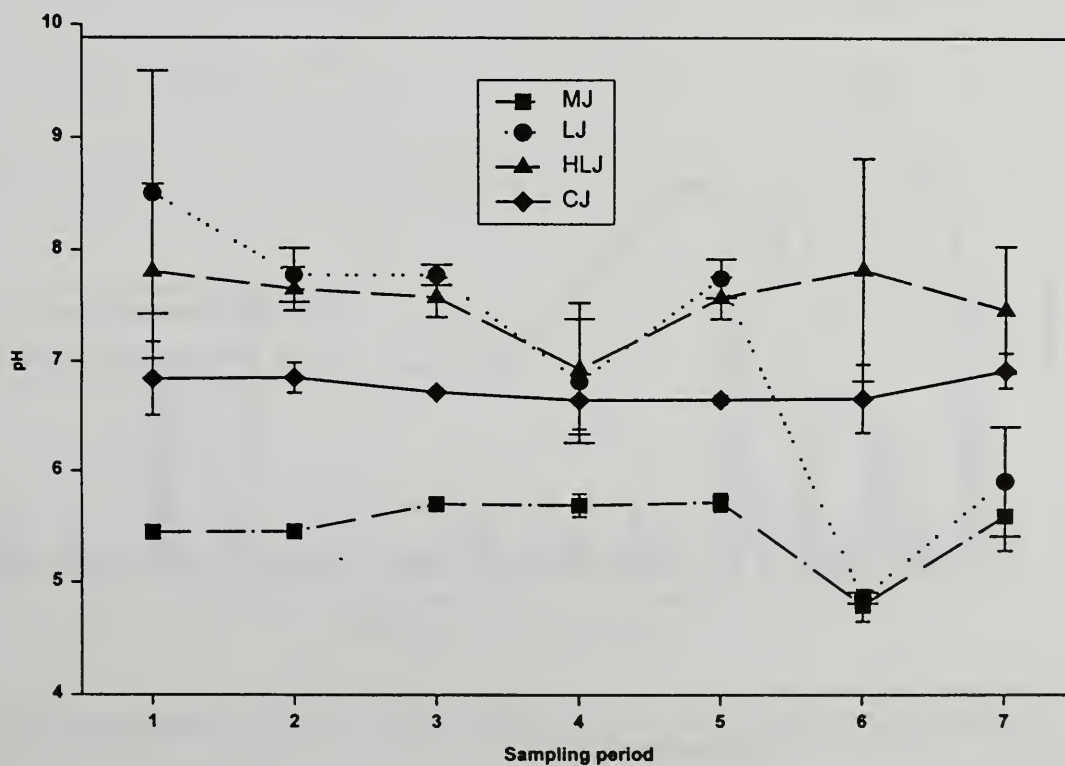


Figure 5. Average pH values across grinding season.

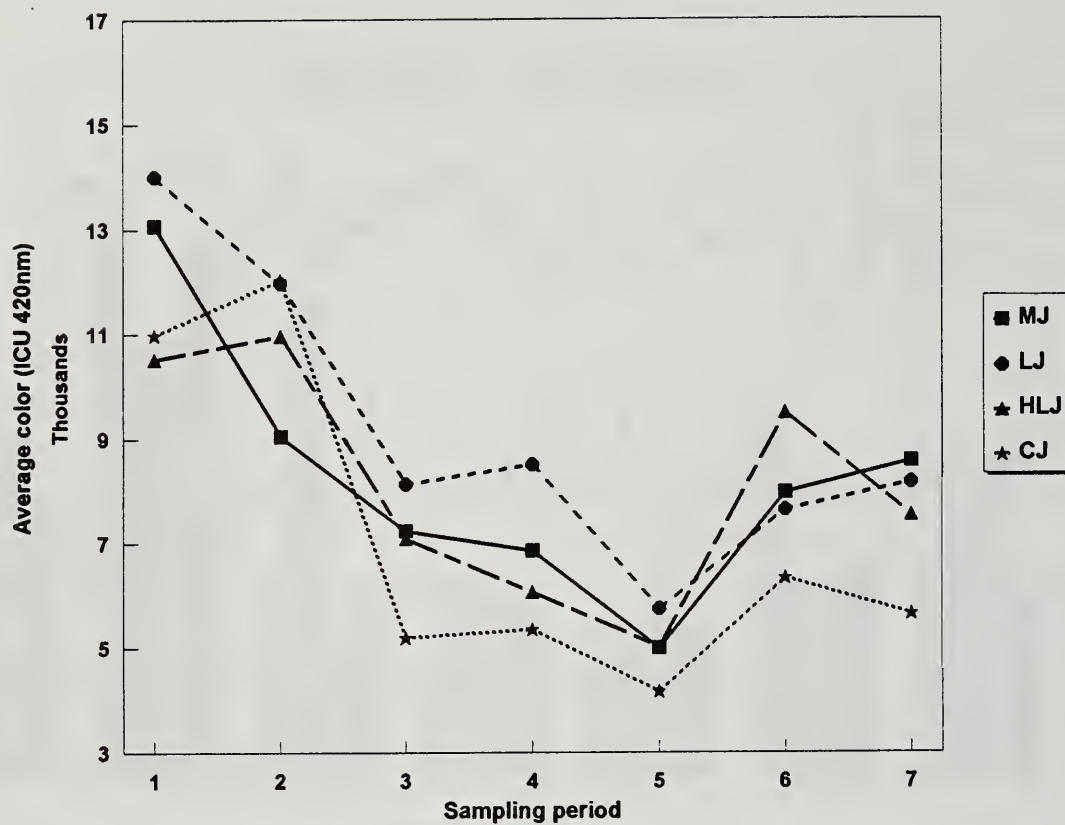


Figure 6. Average color values across the grinding season.

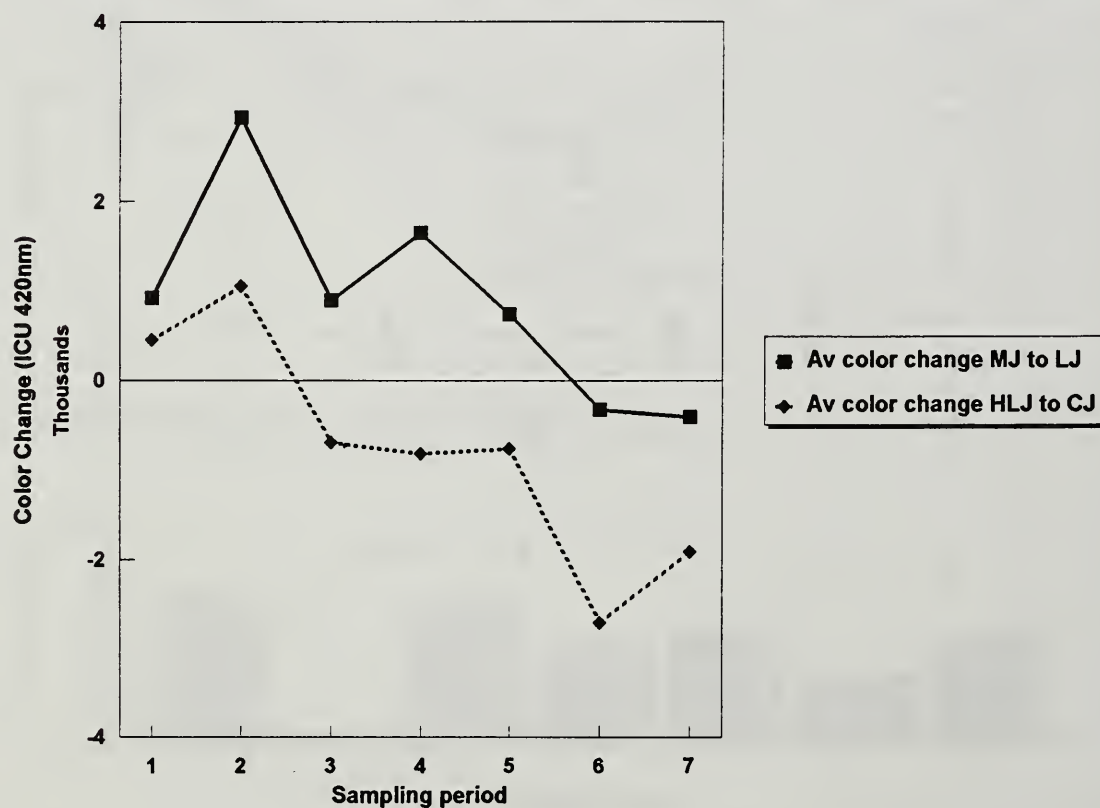


Figure 7. Average color changes across the grinding season.

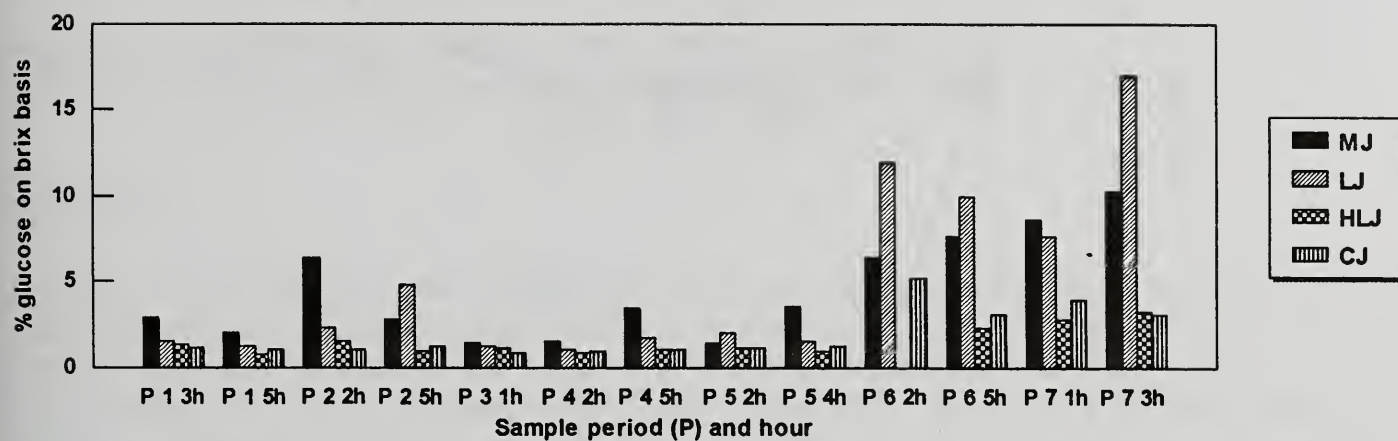


Figure 8. % glucose variation across the grinding season (two random hourly samples).

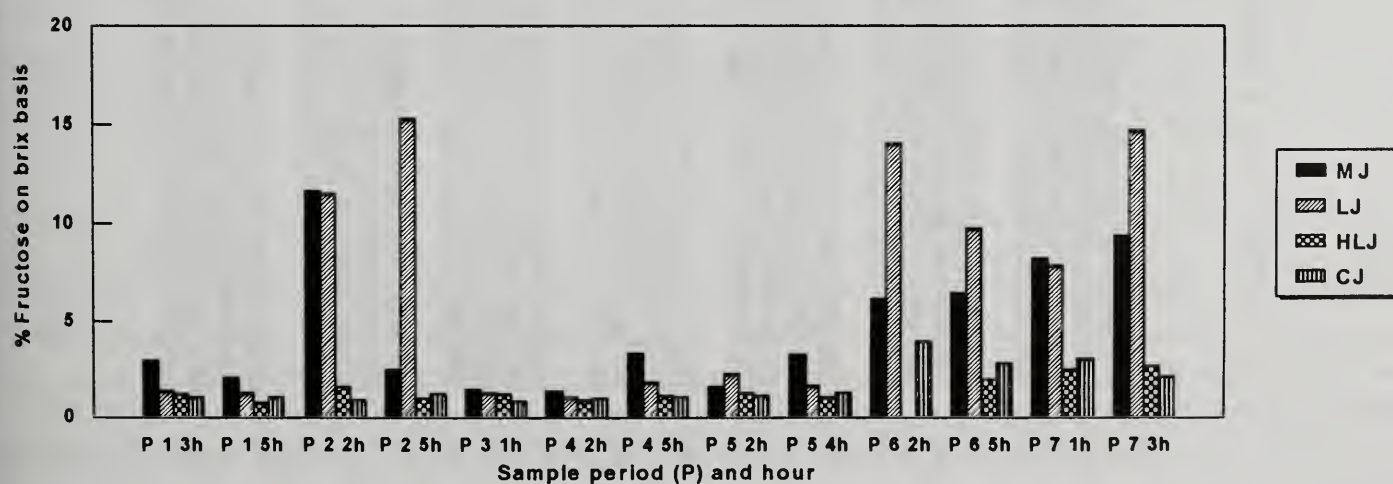


Figure 9. % fructose variation across the grinding season (two random hourly samples).

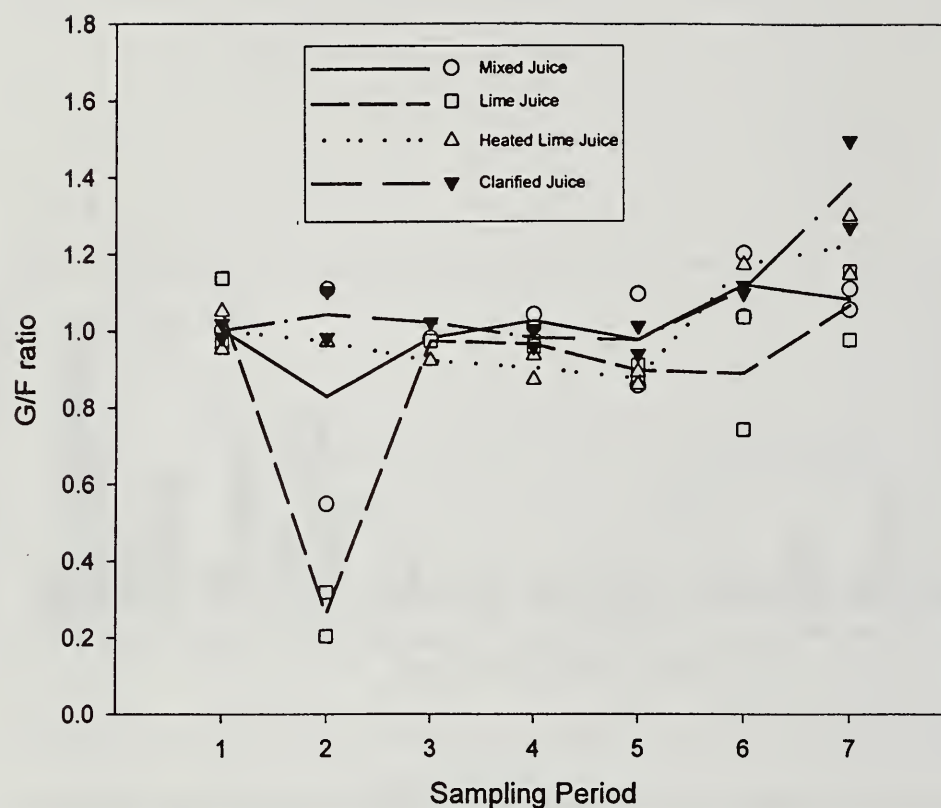


Figure 10. Glucose/fructose ratios across the grinding season (two random hourly samples).

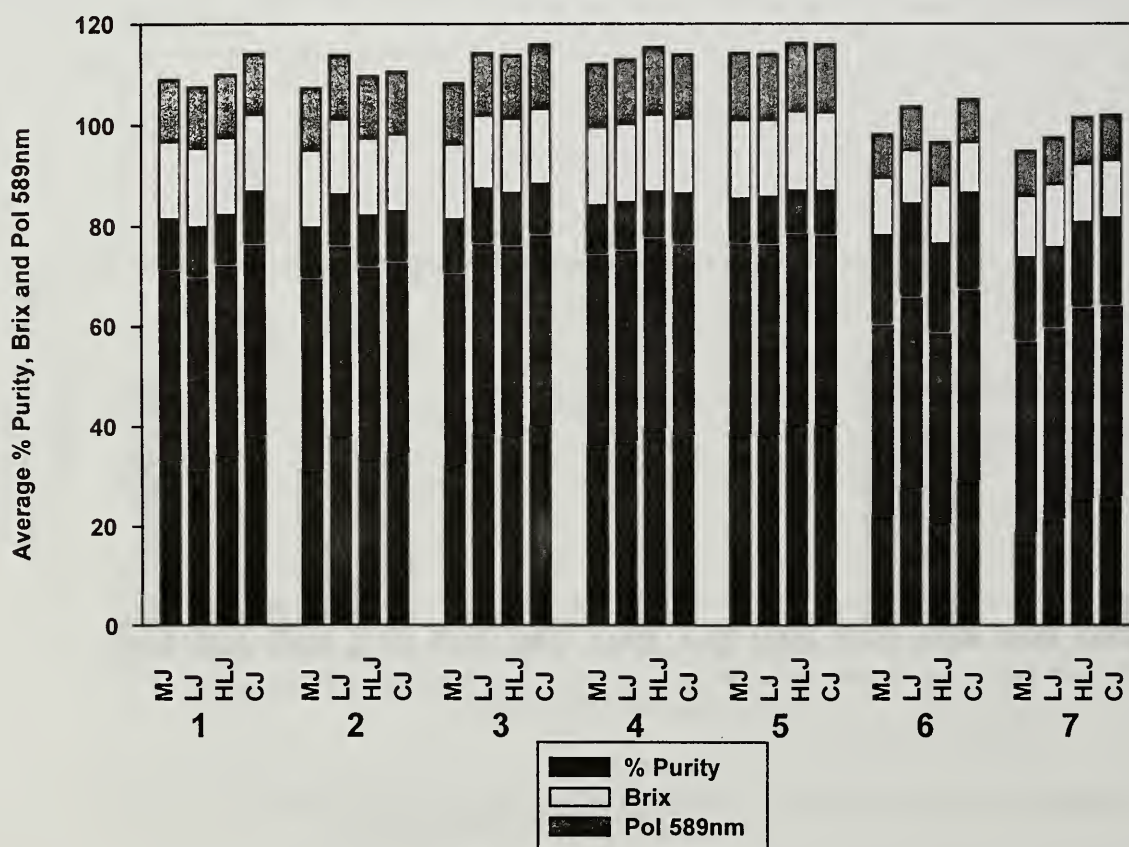


Figure 11. Average purity, Brix and pol values across the clarification process and the grinding season.

POSTER

SURVEY OF ACIDS IN JUICE FROM FREEZE DAMAGED SUGARCANE

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ABSTRACT

At the end of the 1996 crop year, Louisiana experienced a severe freeze that damaged the cane in the experimental plots of the U.S.D.A. Sugarcane Field Unit in Houma, Louisiana. Juice from twelve varieties, some experimental and some cultivated, was obtained for examination of the acids present in juice from freeze-damaged cane. Acids were obtained by liquid-liquid extraction and were identified and measured by GC/MS. Results showed an increased concentration of lactic and succinic acids. Also present were several glycols, including 2,3-butanediol, and increased levels of palmitic (C16:0) acid. These increased acids may contribute to lowered pH and sugar inversion. An increase of lipids may contribute to turbidity in juice and raw sugar.

INTRODUCTION

Louisiana is one of the major cane producing states in the United States. Because the temperate cane growing area of Louisiana is subject to occasional but unpredictable late season freezes, the varieties of cane grown must possess some degree of cold tolerance. Cold tolerance is one of the characteristics selected for in the U.S.D.A. breeding program carried out at the U.S.D.A. Sugarcane Research Unit in Houma, Louisiana. The interest in breeding for cold tolerance in Louisiana dates back to at least 1936 (Brandes, 1939, 1940) and selection of varieties for cold tolerance continues to be an important goal in widely diverse areas of the world where sugarcane is grown (Tai and Miller, 1996; Singh and Singh, 1993).

Various indicators of freeze damage have been used to assess the keeping qualities of varieties after a freeze, including titratable acidity (Irvine, 1963), gum production (Fort and Lauritzen, 1938), ethanol production (South Africa) and changes in Brix, sucrose and purity. Irvine (1965) noted that varieties differed significantly in their normal (pre-freeze) acidity values. Varieties with high pre-freeze acidity tended to have a high post-freeze acidity, and variety evaluations based on titratable acidity alone should be used with caution. Irvine (1971) later observed that soluble polysaccharides (gum) increased slightly in lightly frozen cane and both acids and gums increased significantly when cane was severely frozen.

The molecular basis for cold tolerance in plants is a subject of intense study. Proposed mechanisms include the composition of membrane phospholipids (Lynch and Steponkus, 1987; Raison, 1985) and the presence of antifreeze proteins. Antifreeze proteins are produced endogenously in some plants, including cereals (Antikainen and Griffith, 1997) and genes for antifreeze proteins obtained from polar fishes have been

inserted into tobacco and tomato, (Hightower, *et al.*, 1991). These proteins prevent ice nucleation in plant tissues. Marker compounds for cold tolerance suggested for sugarcane have included peroxidase enzyme (Zhang and Chen, 1993) and the amino acids proline and glycine (Singh, *et al.*, 1992).

Cold tolerance rating for Louisiana varieties is based on six weighted criteria, which are used to develop a cold tolerance rating number, ranging from 1 (susceptible) to 5 (resistant). The criteria used include:

Sucrose	weight = 1	
Purity	weight = 2	
TRS*	weight = 1	(*Theoretical recoverable sugar)
pH	weight = 1	
TA**	weight = 2	(**Titratable acidity)
Dextran	weight = 3	

In this study, juice from severely freeze damaged canes, harvested three weeks after the December 20, 1996, freeze, was analyzed for the type and concentration of acids. A comparison was made to juice from three representative varieties, harvested on December 16, 1997, when there had been no freezes. Additionally, juice from the same varieties pre-freeze (December 18, 1996) was examined.

EXPERIMENTAL

During December 19-21, 1996, sustained freezing temperatures of 24, 22 and 27°F on each night caused severe damage to cane plants. Juice for this experiment was harvested on January 10, 1997, 21 days following the severest part of the freeze. The juice was analyzed for acids and other constituents at the laboratories of Sugar Processing Research Institute, Inc., in New Orleans, Louisiana. The juices had been analyzed for titratable acidity and other quality parameters at the Houma location by established procedures.

Acids were extracted from the cane juice by liquid-liquid extraction of the acidified juice with ethyl acetate: 50 ml juice was acidified to pH 2.50 with HCl and extracted with three 50 ml aliquots of ethyl acetate. The extract was dried over Na₂SO₄ for at least 2 hours, filtered and evaporated to dryness. The dried extract was taken up in 1-2 ml pyridine containing the internal standard, trehalose. A 100 µl aliquot was taken for conversion to the trimethylsilyl derivatives using 0.3 ml TriSil Concentrate. The solution was heated for 10 min at 80°C to ensure complete derivatization.

Identification of constituents was accomplished using a Hewlett Packard Series II Model 5890A gas chromatograph coupled with a Hewlett Packard 5972A mass selective detector (MSD). The MSD was equipped with a hyperbolic quadrupole mass filter and a 70eV electron impact ion source. The column used was 30m x 0.25cm with 0.25µm film thickness, with a DB-5 crosslinked and bonded 5% phenyl 95% methyl silicone phase. Temperature program was 85°C for 4 min; increase 4°C per min to 100°C, then increase 10°C per min to 250°C for 10 min.

Compounds were identified on the basis of their mass fragmentation patterns and retention times. A commercial mass spectral library from Wiley was used for comparison and identification of spectra, along with a S.P.R.I. mass spectral library of standards.

RESULTS AND DISCUSSION

Chromatography revealed a large number of compounds in the juice. These were categorized into carboxylic acids (Table 1a), phenolic acids (Table 2a), fatty acids (Table 3a) and glycerol and glycols (Table 4a). The cold tolerance ratings and titratable acidity values are shown in Table 1a.

Seven carboxylic acids were present in significant concentrations in the 12 varieties examined: Lactic, malonic, succinic, fumaric, malic, aconitic and citric acids. Aconitic acid was by far the major component of the acid fraction, ranging from 1.13 to 4.67% on Brix, followed by lactic acid, which ranged from 1.16 to 0.22% on Brix. While titratable acidity (TA) correlated well with the cold tolerance ratings, no clear correlation emerged with individual acids or total chromatographically measured acids and varieties. The strongest correlation was with lactic acid, when the varieties were pooled and averaged according to cold tolerance rating group, producing a correlation coefficient of -0.9695 ($R^2 = 0.94$). This relationship is shown in Figure 1. Thus, the most cold susceptible varieties would tend to produce more lactic acid. This is in agreement with the finding of Irvine (1971) that gums (mostly dextran) plus acidity give the best correlation with cold tolerance. Both lactic acid and dextran are indicators of microbiological activity, especially that of *Leuconostoc mesenteroides*, a lactic acid and dextran forming bacterium that infects damaged sugarcane.

A comparison of the concentration of acids found in cane juice from three representative varieties from juice taken at the same time of year and level of maturity, in the subsequent year when no freeze had occurred is shown in Table 1b. Figure 2 compares the chromatograms of CP70-321, a cold resistant variety. The chromatograms in Figure 2 as well as the data in Table 1b show a dramatic difference between freeze damaged and undamaged juice. The most significant carboxylic acids in normal, undamaged juice were aconitic, citric, malic and malonic acids. These acids did not change significantly on freezing. Lactic and succinic acids increased significantly, existing in the undamaged juice in only trace quantities. Fumaric acid had about a ten-fold increase, but in either instance, was a minor acid, increasing from 0.002% in undamaged juice to 0.02% in frozen juice. Juice from one day prior to the freeze, December 18, 1996, had similar concentrations of compounds as the juices from December 16, 1997 (data not shown).

The variety NCo-310 had 0.045% citraconic acid. None of the other varieties contained this acid. This variety is more than 35 years old, and not grown commercially in Louisiana, but useful in breeding studies, as a cold tolerant control. NCo-310 is still widely grown in other parts of the world.

Table 2a lists the free phenolic acids identified in the freeze damaged juice. Although not technically a phenolic acid, quinic acid was included in this group since it is often associated with phenolic acids. The concentration of acids in undamaged juice from three representative varieties is shown in Table 2b. The quantity of free phenolics in the freeze damaged juice is very high, compared to the undamaged juice, which was consistently around 60-63 ppm for the three varieties. Syringic acid, ferulic acid and caffeic acid were not found free in the healthy juice.

One of the consequences of freeze damage to tissues is a change in membrane permeability, with leakage of cell components that are normally segregated in vivo. The increase in phenolic acids in freeze damaged cane juice is indicative of this phenomenon. Leakage of phenolic acids from damaged membranes may

contribute, in small part to increased juice acidity, but also may play a major role in increased color formation.

The concentration of palmitic acid (C16:0) and stearic acid (C18:0), the two major fatty acids present in both freeze damaged and undamaged cane juice is shown in Table 3a. Table 3b compares the undamaged juice from the three representative varieties. It is evident that palmitic acid increased several-fold on freezing. The levels of fatty acids were consistent in the three undamaged juices, averaging 140 ppm palmitic acid and 232 ppm stearic acid. The stearic acid changed little in the frozen samples, averaging 289 ppm, but the palmitic acid increased to an average value of 486 ppm. There did not appear to be a correlation with these fatty acids and cold tolerance ratings.

Fatty acids are of interest in cold tolerance research because of speculation that membrane phospholipids and changes therein play a defining role in whether a plant is cold resistant or susceptible. In particular, the increase in the unsaturated C18 fatty acids, linolenic (C18:3), linoleic (C18:2) and oleic (C18:1) are considered a key to cold tolerance (Lynch and Steponkus, 1987).

Oleic and linoleic acid were identified in the extracts of both the freeze-damaged and the normal juices, but in low concentration, ranging around 15-30 ppm for each compound, and not showing any change in frozen versus unfrozen juice. Oleic and linoleic acids have been identified as major components attached to the high molecular weight polysaccharide-colorant complex from fresh cane juice (Godshall, et al., 1998). Research in other plants has shown that as temperatures moderate following a freeze, the level of unsaturated C18 acids decreases again to the non-cold hardy state (Uemura and Steponkus, 1994).

The juice from the frozen varieties all showed significant concentrations of several glycols (Table 4). The compounds eluting at 5.69 and 6.04 min were identified as 2,3-butanediol isomers. The identification was confirmed by standards. (According to the Merck Index, there are three isomeric forms of this compound.) The peaks eluting at 13.29, 13.43 and 13.54 min were tentatively identified as methyl derivatives of 2,3-butanediol by the commercial mass spectral library from Wiley. We did not have standards to confirm this identity. Glycerol determination is also included in Table 4a. As shown in Table 4b and in Figure 2, none of the glycols were present in undamaged cane juice. Glycols could conceivably function as antifreeze compounds as well. However, there was no obvious correlation with glycol concentration and cold tolerance in this study.

The origin of the 2,3-butanediol is unknown at this time, but it appears to be a function of the cold damage to the plant. 2,3-Butanediol is also produced by carbohydrate fermentation with *Bacillus subtilis* (Merck Index) and its presence could be another marker of deterioration similar to lactic acid and dextran. 2,3-Butanediol has also been identified in cold-stressed peanuts (Singleton and Patee, 1997; and Lovegren, 1986), but not in normal or heat-stressed peanuts.

SUMMARY AND CONCLUSIONS

In summary, this study has investigated the presence of carboxylic acids, free phenolic acids, fatty acids and glycols in the juice from 12 varieties of sugarcane, with varying degrees of cold tolerance. The results

showed large increases of lactic acid, succinic acid, palmitic acid and free phenolic acids compared to three unfrozen controls. Only lactic acid correlated with cold tolerance, and this may be related to the increase in gums and dextran that occurs in freeze damaged sugarcane, since the *Leuconostoc* organism responsible for dextran formation is also a lactic acid former. The major acids present in undamaged cane, aconitic, malic and citric did not change on freezing. Malonic acid and stearic acid also showed little change. Unsaturated C18 acids, involved in cold resistance in many plants, did not increase.

Free phenolic acids were much higher in damaged cane juice. These may leak out of cell vacuoles on membrane damage caused by freezing and contribute to the degree of deterioration by causing a color increase and contributing to the acidity.

2,3-Butanediols and substituted glycols (5 peaks in all) were identified for the first time in cane juice. These were present only in the freeze damaged juice. They may function as antifreeze compounds or may be the result of bacterial fermentation.

Differences in membrane lipid composition in a number of graminaceous species have been correlated to cold tolerance, including maize (Pasda and Diepenbrock, 1995), and it would be of interest to conduct a more systematic investigation of membrane lipids in sugarcane varieties.

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Table 1a. Major carboxylic acids found in cane juice from freeze damaged plants. Percent on Brix.

Variety	Cold Tol.*	TA**	Lactic Acid	Malonic Acid	Succinic Acid	Fumaric Acid	Malic Acid	Aconitic Acid	Citric Acid	Total Acids
L65-69	1	4.71	1.16	0.011	0.46	0.02	0.14	4.51	0.11	6.40
CP79-318	1	4.16	0.51	0.006	0.19	0.01	0.18	2.33	0.11	3.33
CP94-552	1	3.89	0.85	0.011	0.63	0.03	0.19	4.67	0.15	6.52
LCP82-89	2	4.12	0.55	0.005	0.40	0.02	0.03	2.66	0.07	3.73
CP72-370	2	3.67	0.90	0.009	0.34	0.02	0.10	3.65	0.09	5.10
LCP86-454	3	3.61	0.93	0.007	0.57	0.02	0.27	3.65	0.22	5.66
CP65-357	3	3.10	0.36	0.006	0.20	0.02	0.16	3.28	0.10	4.13
LCP85-384	3	2.25	0.42	0.009	0.21	0.01	0.18	1.67	0.17	2.66
HoCP85-845	5	2.84	0.22	0.015	0.18	0.04	0.17	1.13	0.15	1.89
LHo83-153	5	2.94	0.53	0.013	0.19	0.02	0.25	3.56	0.13	4.68
CP70-321	5	2.63	0.60	0.009	0.56	0.03	0.11	3.14	0.10	4.54
NCO-310	5	2.67	0.72	0.019	0.61	0.02	0.54	3.23	0.28	5.40

*Cold Tolerance ratings: 1 = susceptible
5 = resistant

**TA = Titratable acidity

Table 1b. Comparison of major carboxylic acids found in cane juice from freeze damaged and undamaged cane plants. Percent on Brix.

Variety	Cold Tol.	Type of Juice	Lactic Acid	Malonic Acid	Succinic Acid	Fumaric Acid	Malic Acid	Aconitic Acid	Citric Acid	Total Acids
CP72-370	2	Frozen	0.90	0.009	0.34	0.02	0.10	3.65	0.09	5.10
		Normal	0.002	0.007	0.003	0.002	0.07	3.43	0.13	3.64
CP65-357	3	Frozen	0.36	0.006	0.20	0.02	0.16	3.28	0.10	4.13
		Normal	0.004	0.11	0.005	0.002	0.13	3.67	0.14	4.06
CP70-321	5	Frozen	0.60	0.009	0.56	0.03	0.11	3.14	0.10	4.54
		Normal	0.002	0.005	0.003	0.001	0.06	2.52	0.10	2.69

Table 2a. Free phenolic acids found in cane juice from freeze damaged plants (ppm on Brix).

Variety	Cold Tol.	p-OH Benzoic	Quinic Acid	Syringic Acid	p-OH Cinnamic	Ferulic Acid	Caffeic Acid	Total Phenolics
L65-69	1	311	79	20	89	39	n.d.	538
CP79-318	1	159	37	30	38	34	19	317
CP91-552	1	123	63	83	58	83	n.d.	410
LCP82-89	2	376	111	n.d.	n.d.	22	n.d.	509
CP72-370	2	75	104	n.d.	53	35	n.d.	267
LCP86-454	3	136	89	n.d.	64	43	19	351
CP65-357	3	393	112	n.d.	129	89	115	838
LCP85-384	3	153	88	21	39	24	6	331
HoCP85-845	5	557	267	22	259	126	264	1495
LHo83-153	5	79	87	23	43	n.d.	17	249
CP70-321	5	n.d.	54	33	55	48	22	212
NCO-310	5	94	406	n.d.	117	37	21	675

Cold Tolerance ratings: 1 = susceptible
5 = resistant

Table 2b. Comparison of free phenolic acids found in cane juice from freeze damaged (top row) and undamaged (bottom row) cane plants (ppm on Brix).

Variety	Cold Tol.	p-OH Benzoic	Quinic Acid	Syringic Acid	p-OH Cinnamic	Ferulic Acid	Caffeic Acid	Total Phenolics
CP72-370	2	75	104	n.d.	53	35	n.d.	267
		8	35	n.d.	17	n.d.	n.d.	60
CP65-357	3	393	112	n.d.	129	89	115	838
		7	36	n.d.	20	n.d.	n.d.	63
CP70-321	5	n.d.	54	33	55	48	22	212
		4	37	n.d.	22	n.d.	n.d.	63

Table 3a. Fatty acids in juice from freeze damaged plants (ppm on Brix).

Variety	Cold Tol.	Palmitic Acid	Stearic Acid	Total Fatty Acids
L65-69	1	424	239	663
CP79-318	1	431	260	691
CP91-552	1	615	281	896
LCP82-89	2	401	300	701
CP72-370	2	468	346	814
LCP86-454	3	758	452	1210
CP65-357	3	359	132	491
LCP85-384	3	315	216	531
HoCP85-845	5	524	395	919
LHo83-153	5	380	257	637
CP70-321	5	567	281	848
NCO-310	5	587	303	890

Cold Tolerance ratings: 1 = susceptible
5 = resistant

Table 3b. Comparison of fatty acids found in cane juice from freeze damaged and undamaged cane plants (ppm on Brix).

Variety	Cold Tol.	Type of juice	Palmitic Acid	Stearic Acid	Total Fatty Acids
CP72-370	2	Frozen	468	346	814
		Normal	124	219	343
CP65-357	3	Frozen	359	132	491
		Normal	164	263	427
CP70-321	5	Frozen	567	281	848
		Normal	131	215	346

Table 4a. Total glycerol and glycols in juice from freeze damaged cane plants. Percent on Brix.

Variety	Cold Tol.	5.69 min*	6.04 min*	Glycerol	13.29 min**	13.43 min**	13.54 min**	Total Glycerol + Glycols
L65-69	1	0.079	0.508	0.008	0.020	0.150	0.183	0.95
CP79-318	1	0.150	0.325	0.007	0.003	0.017	0.023	0.53
CP91-552	1	0.135	1.040	0.010	0.016	0.149	0.148	1.50
LCP82-89	2	0.153	0.896	0.008	0.019	0.134	0.134	1.34
CP72-370	2	0.082	0.520	0.008	0.013	0.099	0.106	0.83
LCP86-454	3	0.266	1.087	0.010	0.011	0.081	0.074	1.53
CP65-357	3	0.106	0.480	0.006	0.006	0.039	0.038	0.68
LCP85-384	3	0.040	0.215	0.006	0.004	0.026	0.025	0.32
HoCP85-845	5	0.047	0.188	0.022	0.015	0.018	0.033	0.32
LHo83-153	5	0.023	0.137	0.007	0.004	0.021	0.026	0.22
CP70-321	5	0.170	1.043	0.097	0.017	0.140	0.134	1.58
NCO-310	5	0.191	0.983	0.024	0.005	0.022	0.021	1.25

Cold Tolerance ratings: 1 = susceptible
5 = resistant

* Isomers of 2,3-butanediol

**Possibly methyl substituted forms of 2,3-butanediol

Table 4b. Comparison of glycerol found in cane juice from freeze damaged and undamaged cane plants. (Glycols not detected in undamaged juice.) Percent on Brix.

Variety	Cold Tol.	Type of juice	Glycerol
CP72-370	2	Frozen	0.008
		Normal	0.002
CP65-357	3	Frozen	0.006
		Normal	0.002
CP70-321	5	Frozen	0.097
		Normal	0.002

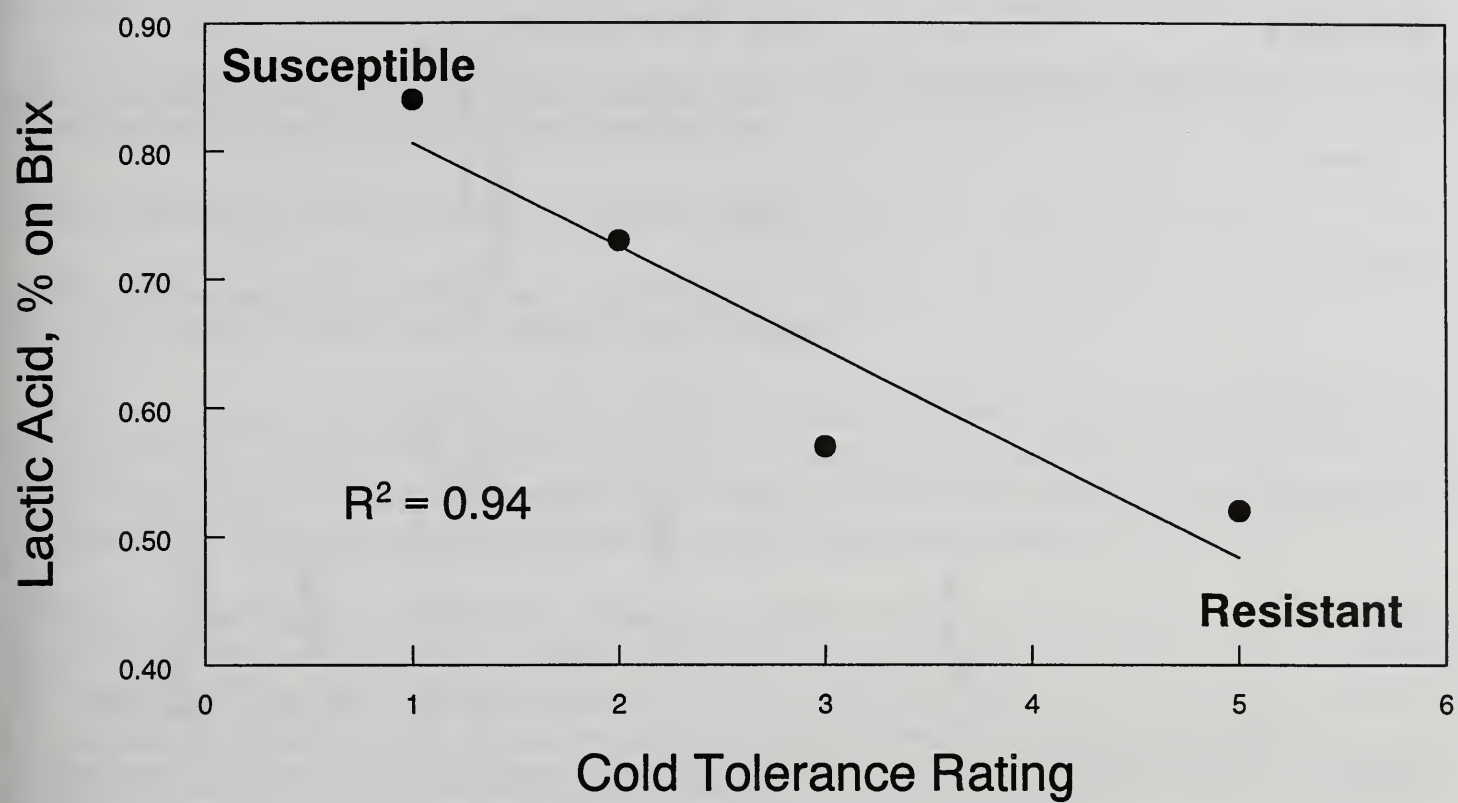


Figure 1. Lactic acid and cold tolerance in cane varieties.

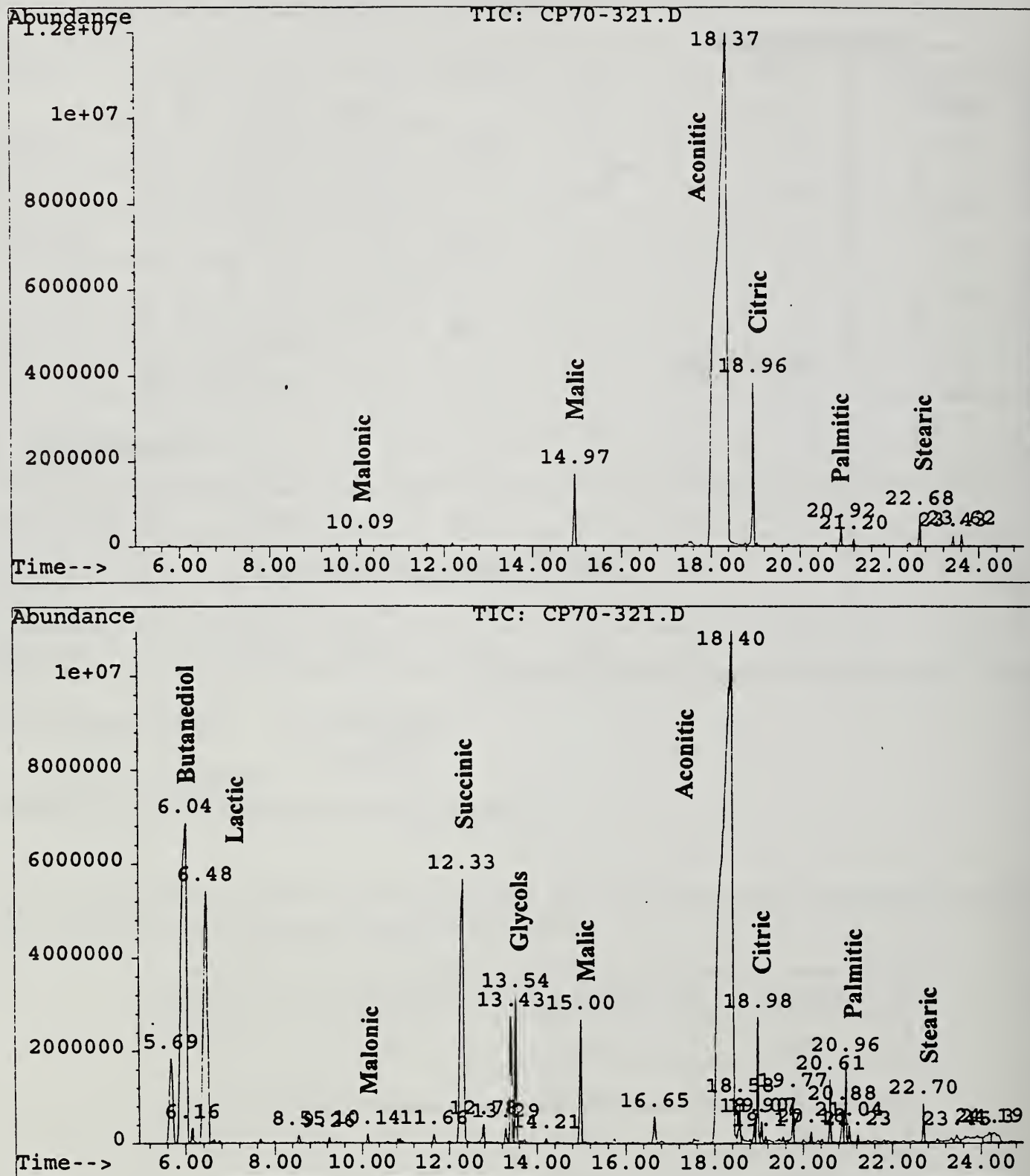


Figure 2. Top: Extract of undamaged cane juice from variety CP 70-321, a cold tolerant variety. Bottom: Extract of freeze damaged juice from the same variety.

POSTER

DEGRADATION OF COLOURANTS EXISTING IN A SUGAR REFINERY EFFLUENT BY THE FUNGUS *PHANEROCHAETE CHRYSOSPORIUM*

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Colour in the sugar industry consists of a complex mixture of different types of colourants, the most important being: 1) phenolic compounds, coming from the cane plant, 2) caramels, which are produced by thermal degradation and condensation reactions of sugars, 3) melanoidins, formed from sugar-amino acid reactions via the Maillard reaction and 4) hexose alkaline degradation products (HADPs) (1).

During the refining process, part of the colourants is removed from the sugar liquor by anion-exchange resins. The pre-regeneration of these resins is done with 50 g/l NaCl, giving rise to an effluent containing those types of colourants. This effluent presents an environmental problem due to the presence of phenolic compounds, intense colouration and high organic load (COD). The organic load can be eliminated, at least in part, using traditional biological treatments but the compounds responsible for the intense colouration are poorly degraded by the organisms normally involved in these treatments (2,3).

The white-rot fungus *Phanerochaete chrysosporium* is a potentially useful microorganism in waste treatment systems because it is able to degrade a broad spectrum of structurally diverse organic compounds. Evidence suggests that the unique ability of *P. chrysosporium* to degrade those compounds is due, at least in part, to the lignin degrading enzymatic system of this microorganism that is non-specific and partially extracellular (4,5).

In this work we investigated the activity of *P. chrysosporium* towards each of the four main types of colorants present in the sugar refinery effluent. It was observed that the fungus was able to decolourise melanoidins, caramels and HADPs solutions by 74%, 87% and 80%, respectively, and to reduce phenolic compounds by 72%. Gel permeation chromatography studies showed that decolourisation was accompanied by an effective degradation of the colorants and not only a transformation of the visible chromophores and that a slight polymerisation of phenolic compounds and a depolymerisation of melanoidin also occurred after incubation. Furthermore, the resulting products of fungal action proved to be non-toxic.

In summary, we demonstrated that *P. chrysosporium* was able to degrade the four main types of colorants present in the pre-regeneration effluent: phenolic compounds, caramels, melanoidins and HADPs. Therefore, *P. chrysosporium* seems to be a potentially useful microorganism in the treatment of effluents from sugar industries and other related industries (e.g., fermentation industries using molasses).

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POSTER

MICROSCOPICAL EXAMINATION OF INSOLUBLE MATERIALS FROM SUGAR PROCESSING

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ABSTRACT

Insoluble materials that may be initially present or introduced during the various stages of sugar processing ultimately affect the quality of the finished product. Imaging by scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDS) can assist in the identification of insoluble materials found in sugar. Samples from several types of sugars showed the usefulness of both of these techniques. Specific methods, such as centrifugation, dialysis, and filtration, were used to separate the insoluble materials from the sugar samples. The separated particles were studied microscopically by SEM and analyzed chemically by EDS to obtain elemental composition. Examples of the isolated materials included fibers, crystals, microorganisms, and plant materials. EDS analysis found silicon, calcium, phosphorus, iron, and aluminum in these particles. The combination of these techniques can provide the sugar processing industry with the means to identify sediments in sugar samples and suggest methods to alleviate them.

INTRODUCTION

Traces of insoluble materials are often found in sugars at various stages of processing. Identification of these materials provides information necessary for devising methods for their removal, and to ensure that they are not harmful to the product. Because insolubles are often present in small quantities, and because their individual sizes are often microscopic, microscopical methods are usually necessary for their identification and characterization (1). The scanning electron microscope (SEM) produces images of magnification great enough that individual particles can be examined. Observations at these increased magnifications provide information to identify the materials. When direct visualization cannot identify the particle, a correlative elemental analysis technique, energy-dispersive x-ray analysis (EDS), can usually identify elements present, and thus suggest the composition of the materials (2).

METHODS

The specific procedures describing this methodology are presented in a separate paper published in this Proceedings (3). Insoluble materials were separated from sugar solutions by centrifugation, dialysis, or filtration. A 0.45 μ cellulose acetate filter was used in the filtration process to separate sugar sediments. Sugar samples were centrifuged at 2500 rpm for ten minutes. One to two drops of the centrifuged sample were placed on carbon planchets after adhering them to standard SEM stubs with double-stick tape. After

air-drying the sample, the stub was then carbon coated by vacuum evaporation techniques to suppress charging and eliminate any elemental interference with EDS acquisition. The dialyzed and filtered samples were mounted directly onto these stubs before vacuum evaporation. The samples were ready for SEM and EDS identification.

RESULTS AND DISCUSSION

SEM comparisons of the three techniques used to isolate insoluble materials from the sugar samples showed centrifugation techniques to be most effective. Particles were sufficiently well isolated on the sample stubs allowing for meaningful analysis by EDS. Elemental spectral maps and analog dot maps of individual elements were obtained along with comparisons of different sugar samples by SEM and EDS (Figures 1-4). Similar comparisons were made between centrifuged and dialyzed samples of the same sugar (Figures 3-4). Some samples contained an amorphous material, probably a polysaccharide, which dried and left a coating of film over the particulate material. The film hindered identification of the particles by masking structural characteristics and limiting elemental analysis. This effect was more evident with dialyzed samples. Extensive washing and centrifugation of the dialyzed samples was necessary to remove most of the film from the sample before mounting procedures could proceed.

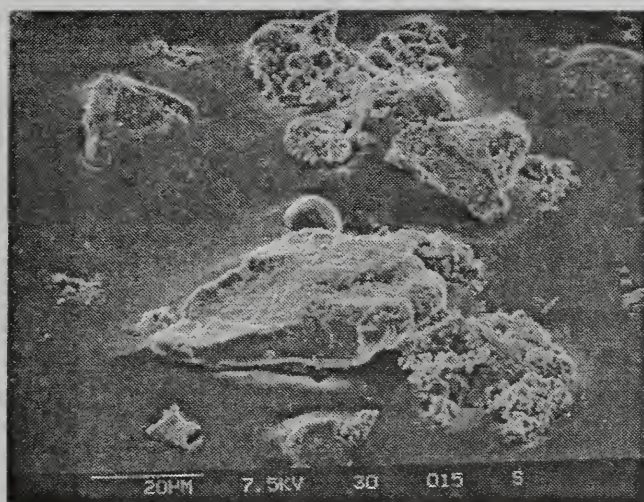
Imaging of the filtered particles was complicated by the pore size of the background support medium which interfered with visualization. Also, charging of the sample, where bright spots or streaks on pictures indicate poor sample coating conductivity (Figure 5), reduced the effectiveness of this sample preparation method. Bacterial sample contamination (Figure 6) was evident when some of the centrifuged samples were imaged. Consequently, samples were kept frozen after all centrifugation processes although this contamination may have occurred in the field or during processing.

CONCLUSION

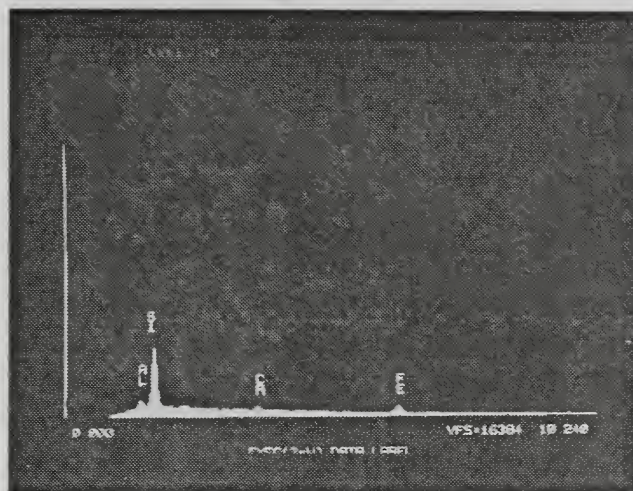
SEM imaging and EDS spectroscopical analysis are important techniques for both observing some of the particulates found in processing sugar samples and providing elemental descriptions of these contaminants.

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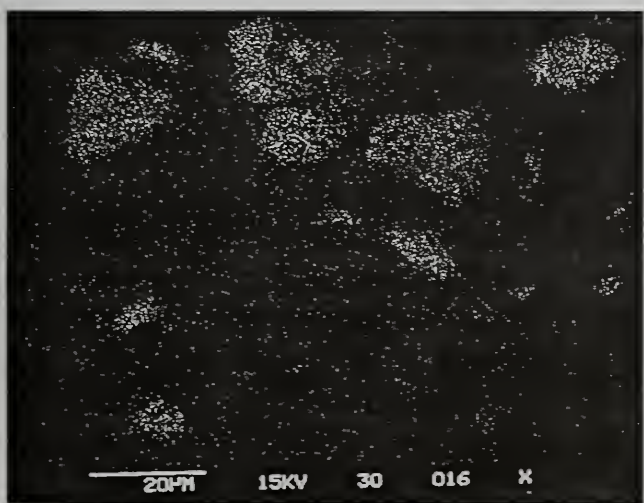
1. Cleriot, J. (1994). Sugar manufacture seen through the microscope, *Int. Sugar J.*, 96 (1144): 129-142.
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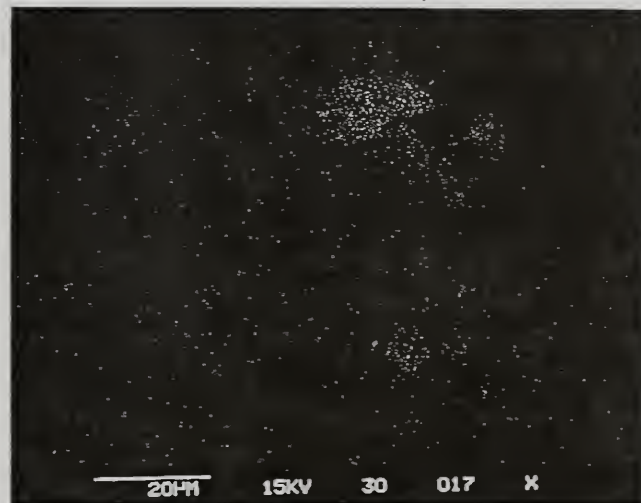
{A} SEM Image, 1000x



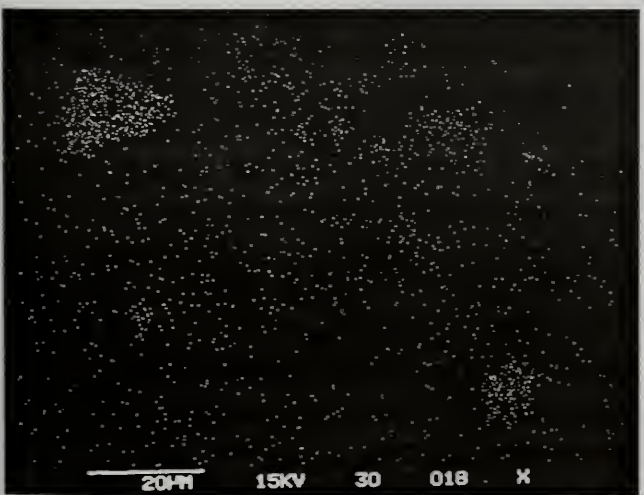
{B} EDS Spectrum, 1000x



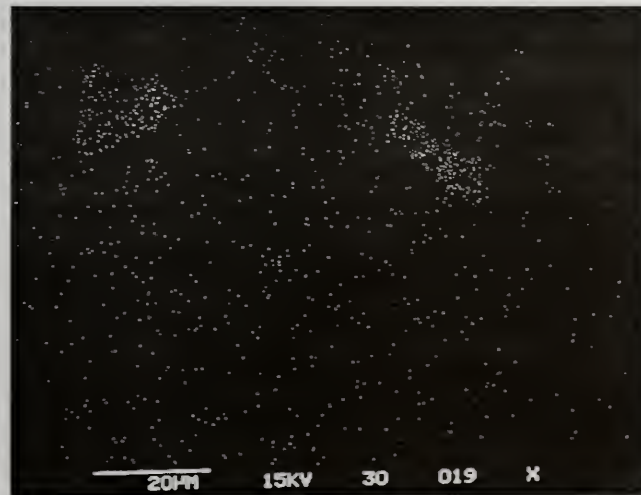
{C} Silicon Dot Map, 1000x



{D} Iron Dot Map, 1000x

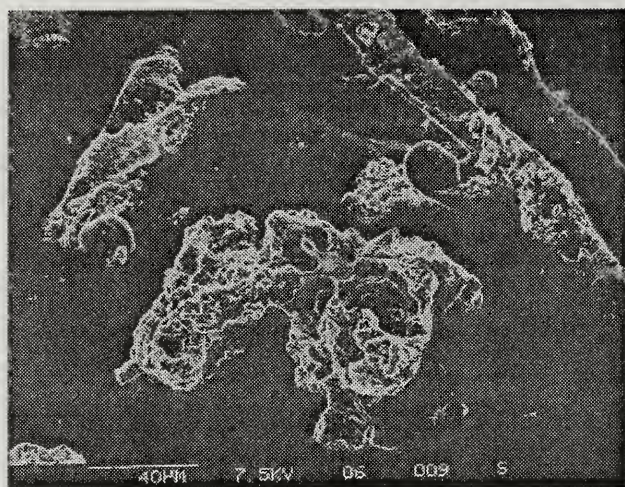


{E} Aluminum Dot Map, 1000x

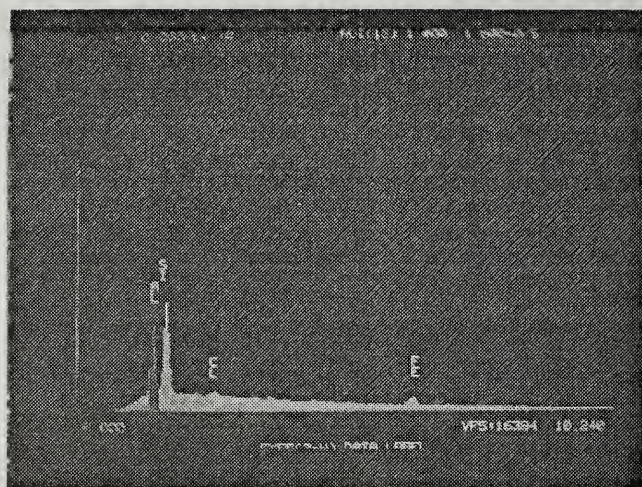


{F} Calcium Dot Map, 1000x

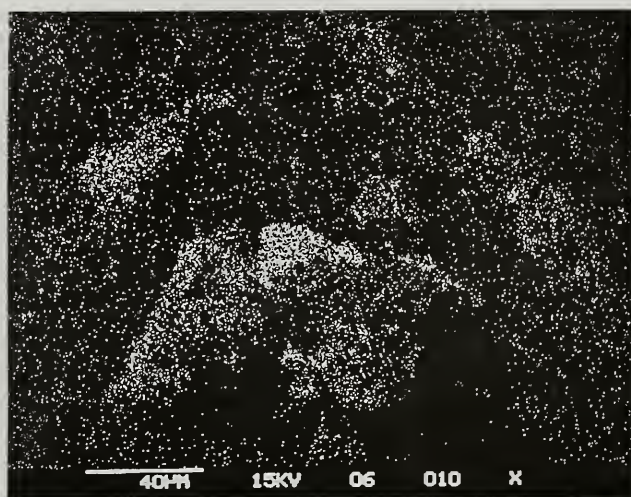
Figure 1. White sugar beet sample, centrifuged, air-dried on stub and carbon coated.



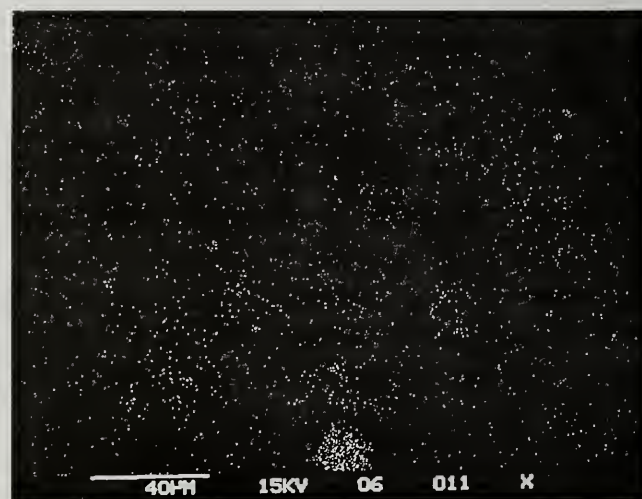
{A} SEM Image, 500x



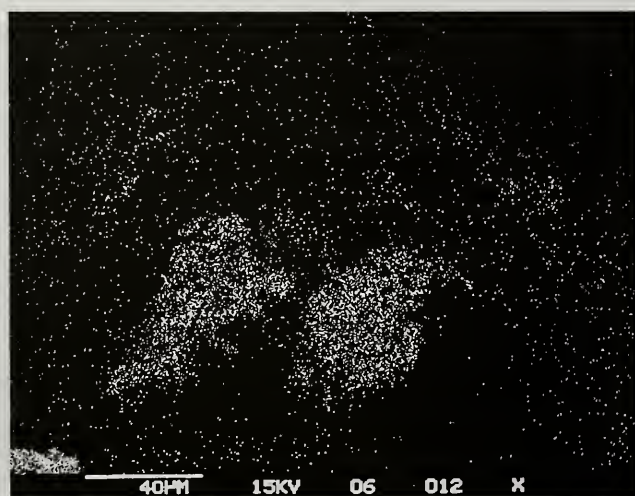
{B} EDS Spectrum, 500x



{C} Silicon Dot Map, 500x

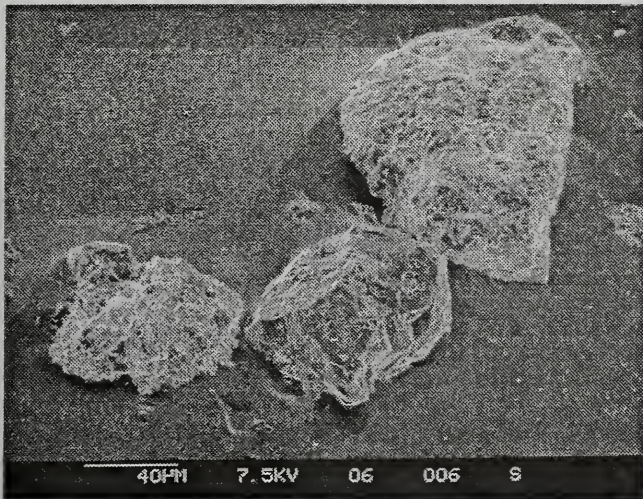


{D} Iron Dot Map, 500x

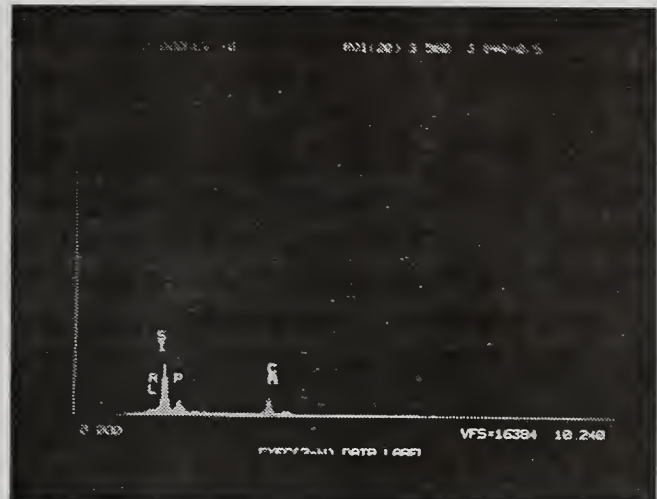


{E} Aluminum Dot Map, 500x

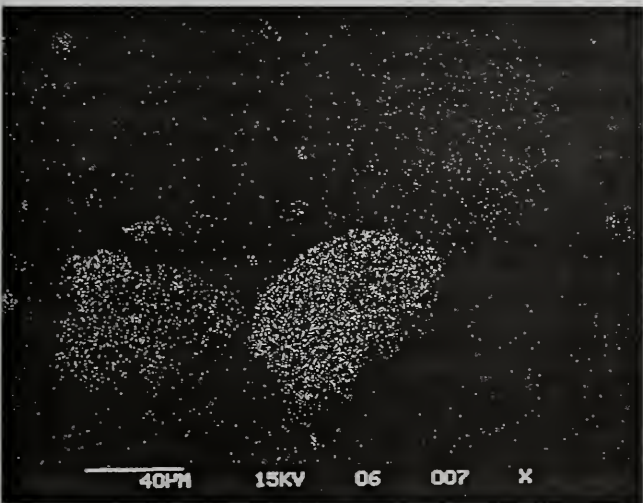
Figure 2. Refined sugar cane sample, centrifuged, air-dried on stub and carbon coated.



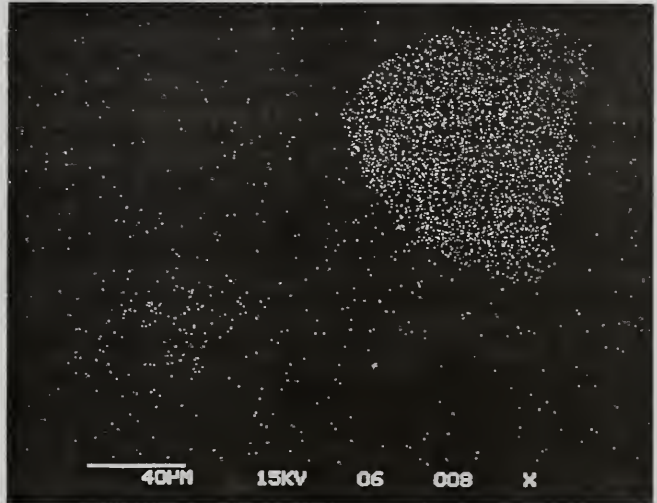
{A} SEM Image, 400x



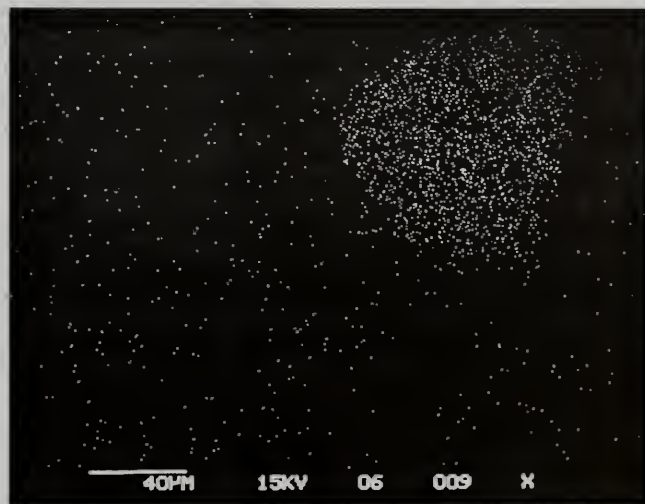
{B} EDS Spectrum, 400x



{C} Silicon Dot Map, 400x

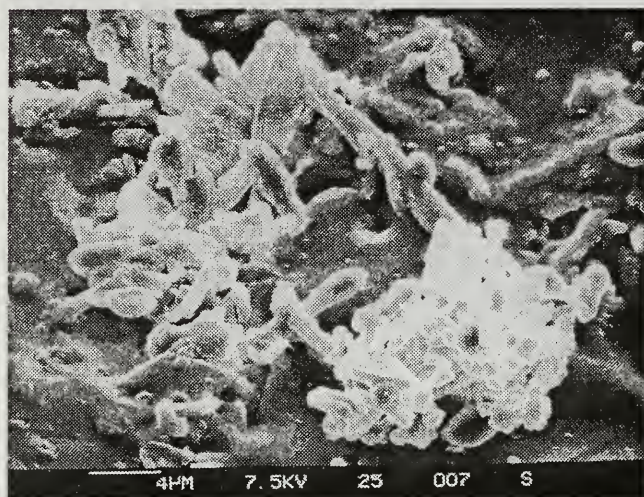


{D} Calcium Dot Map, 400x

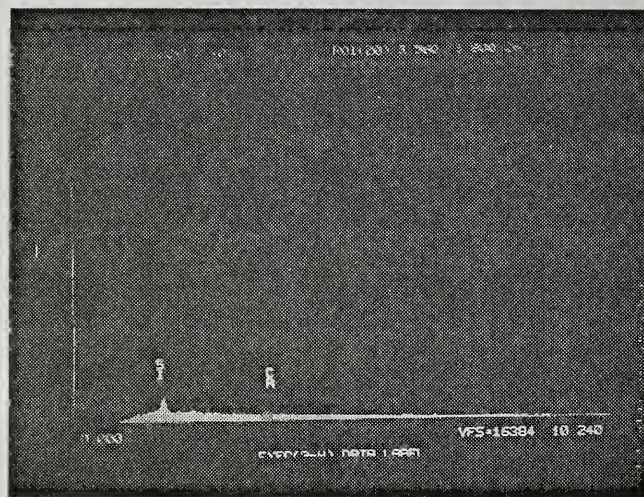


{E} Phosphorus Dot Map, 400x

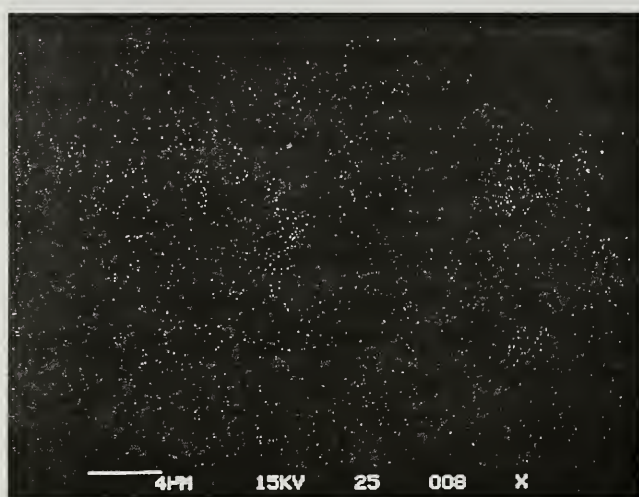
Figure 3. Raw sugar cane sample, centrifuged, air-dried on stub and carbon coated.



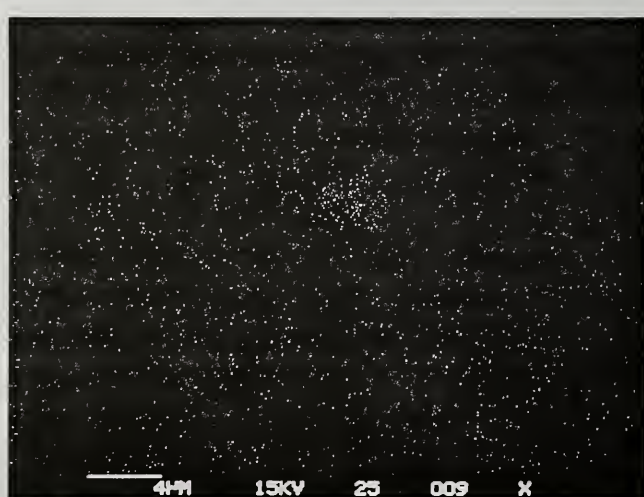
{A} SEM Image, 3000x



{B} EDS Spectrum, 3000x

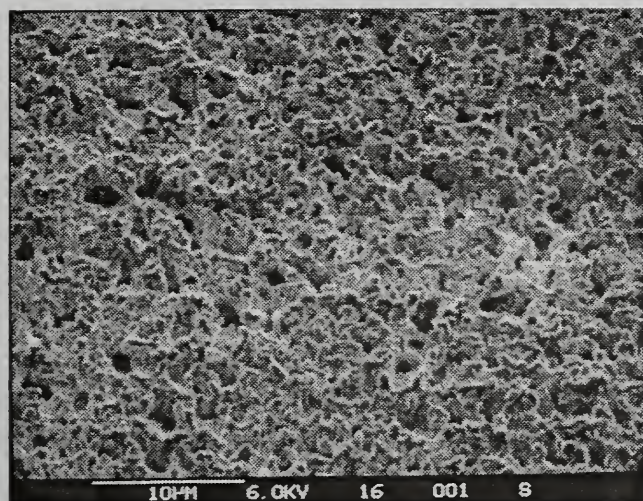


{C} Silicon Dot Map, 3000x



{D} Calcium Dot Map, 3000x

Figure 4. Raw sugar cane sample, dialyzed, air-dried on stub and carbon coated.



{A} SEM image of cellulose acetate filter paper control, 2500x.

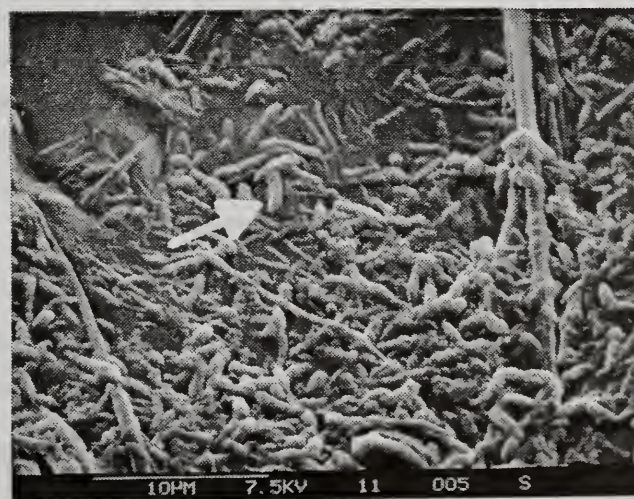


{B} SEM image of filtered particles on cellulose acetate filter paper, 2000x. Particles show "charging" of the sample and difficulty in distinguishing them from the background material.

Figure 5. Example of filter paper extraction method of sugar samples.



{A} Low magnification SEM image, 800x. Centrifuged sample air-dried on stub and carbon-coated.



{B} High magnification SEM image, 3000x. Centrifuged sample air-dried on stub and carbon-coated.

Figure 6. SEM images of sugar samples contaminated with bacteria.

POSTER

ON-LINE COLOR MEASUREMENT OF RAW AND WASHED RAW CANE SUGARS

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INTRODUCTION

Measurement of sugar colour is important for product quality control and for process control in refineries and factories that produce white sugar. In North America, the colour measurement in solution form (either ICUMSA colour (4) or Reference Base Units) provides common ground between sugar manufacturers and their customers. At present, there is no measurement of colour in solid crystalline white sugar that is widely used for white or raw sugars. Reflectance measurement is available (4) (Braunschweig colour standards) and is in use in Europe, but is not in wide use in North America, because it correlates poorly to the established solution colour measurements.

Colour measurement is important in process control as a measure of process efficiency and of product quality. Colour content correlates roughly with ash, moisture and non-sugar content; there are rapid tests for ash and moisture, but not for colour. Colour of final product is almost always included in customer specifications. Indeed, in recent years a specification based on ICUMSA colour has been added to white sugar contracts traded on the futures market.

Sugar of colour above specifications must be recycled in the refinery or factory at considerable cost, or may be returned by the customer at even more expense. In production of crystal sugar, good controls are available for crystallisation, and size and yield of crystals. However, for the subsequent separation of sugar crystals by centrifugation, and washing and drying, few controls or tests are available without expensive use of labour. And so the final preparation of white sugar, at its most cost-sensitive stage, is run without frequent monitoring or inspection.

A rapid, reproducible on-line measurement for colour in solid sugar, that correlates to the accepted solution colour, is desirable. A system to make such a measurement has recently become available. The multiwavelength instrument from Neltec Denmark can measure colour of sugar in less than 5 seconds, on a moving belt or conveyor, with no sample preparation (1,2). The instrument can be used to measure colour, in terms of up to 5 different calibrations, in wet or dry sugar, in a screw or hopper conveyor or on a belt.

The system has been installed on white sugar lines to check colour of wet white sugar coming from centrifugals (1,3), so that the performance of each centrifugal can be monitored automatically.

Advantages of this system are:

1. The risks of bad sugar in the drier or the silo are eliminated, as is the risk of inadvertently shipping sugar with too high a colour value.
2. Better control of centrifugals. Malfunctioning is reported immediately. As it is possible to trace which centrifugal is not working properly, response can follow without delay. Stops in the production due to centrifugal problems can be avoided or the consequences can be greatly reduced.
3. Trimming of washing times. The fast feed-back on changes in washing times for each centrifugal makes trimming easy. With continuous monitoring of quality, the security distance to the quality limit can be reduced, leading to energy savings.
4. Speed. The results are presented in less than 5 seconds.
5. No sample preparation is required. The measurements are taken directly in the production stream. The frequency of the laboratory tests can be reduced, with labour savings.
6. The quality is improved. More uniform quality is better quality.

In the present paper, successful applications of the Neltec system of colour measurement of raw cane sugars and of soft brown or yellow sugars are reported. Applications to white sugar colour measurement are reviewed. In the case of soft sugars, calibrations can be made against ICUMSA colour or against colour from the AGTRON™ measurement that is popular in North America, to give a continuous analysis of soft sugar colour, one of the major customer specifications for soft sugars. The system can be applied to boiled soft sugars or to those prepared by a coating and blending process.

Raw sugar colour can be measured continuously on raw sugar entering a refinery, or, and perhaps more significantly, on washed raw sugar coming out of the affination centrifugals. Until now, there has not been (to the authors' knowledge) a system for continuous measurement of washed raw sugar colour, which is probably the most important parameter for process control in a refinery. The colour of washed raw sugar, coming out of the centrifugals, can be monitored every few seconds by the Neltec system.

METHODS AND MATERIALS

Measurement System

The basic measurement unit works on a very simple principle (Figure 1). An illuminator sends out short pulses of white light. A detector collects some of the reflected light and separates it into spectral components. Both units have housings of stainless steel, and are placed at a distance of about 1 meter from the sugar. Therefore they can resist the environmental conditions, and are not contaminated by the sugar.

Information about the reflected light is sent from the detector to a computer, where the colour is calculated and presented to the user. Two graphs are used, a short term graph showing colour during the last 30 minutes (Figure 2), and a long term graph to show the last 24 hours (Figure 3). Under the short term graph

is shown information about which centrifugal(s) has delivered sugar to the measurement position at the time of measurement (Figure 4). In this way the operator is able to identify centrifugals with unsatisfactory function.

Calibration of the ColourQ 800

The system must go through two calibrations, one initial and one check calibration at regular intervals. The initial calibration is made after the installation. A number of samples are taken from the normal production stream. Their colours are determined in the laboratory. These results are combined with the measurements from the system to give a calibration curve. This is then implemented in the system. Later during normal operation a check calibration is needed to compensate for drift in the instrument. This is made simply by measuring a white tile at regular intervals.

Laboratory Installation and Operation

Although the Neltec ColourQ 800 is designed to operate in a factory environment with sugar moving on conveyor belts or screws, the laboratory set up for calibration development, validation and precision tests used stationary sugar samples. The Neltec light source and detector were mounted on a scaffold (Figure 5) above a sample tray (25 cm wide x 75 cm long x 7 cm deep with a white enamel inside surface facing the detector). The white sugar samples were loaded onto the tray with a device that simulates sugar falling onto a conveyor belt. For raw sugars and soft sugars a brown paper liner was placed inside the sample tray and the samples were loaded by hand. The surface of the sample was between 70 and 85 cm below the light source and detector.

Calibration and Validation at S.P.R.I. Laboratories

Calibrations for raw sugar were developed from Neltec measurements and conventional colour measurements (ICUMSA, both buffered and old unbuffered methods) obtained at S.P.R.I. Calibrations for refined cane sugar were developed from Neltec measurements obtained at S.P.R.I. and conventional colour measurements (ICUMSA, buffered method) obtained from the refinery supplying the sugar. Calibrations for soft sugar were developed from Neltec measurements obtained at S.P.R.I. and conventional colour measurements (ICUMSA, buffered and old unbuffered methods) obtained at S.P.R.I. and AGTRON™ colour measurements obtained from the refinery supplying the sugar.

Multiple linear regression or neural network methods were used to develop the calibration models. The validation data was obtained at S.P.R.I.

RESULTS

Details on the data sets used to develop the models are shown in Table 1.

A. White sugars - dry

In a production environment the sugar is measured on a moving conveyor belt, where it is measured as a continuous flow with an even surface. In the laboratory measurements that surface is less even, because the sugar is loaded on the sample tray by a hand-operated device. To test the influence of this the validation was repeated.

The results are shown in Table 2 and Figure 6. The figure has data from the first validation only. The average colour measured by the refinery was 39.0, versus 37.5 and 39.3 by the two validations.

It can be observed that the precision of the laboratory measurements (SEP 3.1 and 2.8 by the two validations) is not as narrow (SEP 2.23) as reported (3) for measurements in a production environment. Apparently the smaller sample size and the hand loading have a negative effect on the precision of the measurement. The precision of the production on-line measurement compares very well with the repeatability of the ICUMSA solution colour measurement of 3 ICU (repeatability: same operator, same sample) (4).

B. Raw sugars

The calibration was made on the S.P.R.I. Raw Sugar Library on sugars with ICU up to 15,457.

The results are shown in Figure 7. The average of the ICU is 4635 and 4992 of the prediction. SEP is 933. The SEP appears to be high; in fact, for 13 of the 89 samples the Neltec and ICUMSA colour differed by more than 1,000 ICU (residuals > 1,000 ICU) and, of course, these samples inflate the SEP value. It is important to note that in this and other studies a visual ranking of raw sugar colour intensity often does not agree completely with a ranking by the ICUMSA measurement. This difference is related to the proportions of various classes of colorants in raw sugars (7,8) and to the sensitivity of the human eye to light at 420 nm; a more detailed explanation is beyond the scope of this report. Comparison of ICUMSA and visual ranking of the raw sugar library indicated all samples that ranked differently had Neltec-ICUMSA residuals > 1,000 ICU. That is, that in every case when the Neltec measurement had > 1,000 ICU difference from the ICUMSA colour value, the visual colour ranking disagreed with the ICUMSA ranking. Furthermore, the ranking of raw sugar colour by the Neltec agreed with the visual ranking rather than with the ICUMSA ranking.

The S.P.R.I. library of raw sugars covers the widest possible range of quality, colour and grain size, from very light VHP (Very High Purity; highly washed) sugars with minimum syrup coating, to fine grain sugars with a lot of molasses coating that have been stored for years. The error in ICUMSA colour measurement on this set is much greater than the error of 200 to 400 on a set of narrower range (eq. 6000 to 8000 ICU). Sources of the ICUMSA error in measurement of raw sugar colour are many in addition to difference in crystal coating. The filtration step filters out a non-uniform amount of colour from each sugar (5,6). The higher molecular weight colour is more likely to be filtered out on a Millipore-type membrane. However, this is the very colour that continues to persist into the refined sugar crystal, because no refinery filtration will simulate the membrane effect.

Errors in the raw sugar colour measurement by ColourQ have to have the same range for accuracy as the ICU measurements, since the Neltec System is calibrated against ICU, but the ColourQ shows much better precision than the ICUMSA measurement, as shown in Table 3. Repeatability (precision) is an important factor in the continuous measurement of raw sugar colour.

Because the Neltec system can be used on wet or dry sugars, it is proposed that the system be used to measure colour of washed raw sugar coming out of the affination centrifugals. Trials on this proposed system are now taking place. Colour of washed raw sugar is a most important control factor for any refinery; knowledge of WRS colour allows predictions for control of clarification and decolorisation. To the authors' knowledge, there is no continuous monitor for washed raw sugar colour currently in use. Most refineries take this measurement once every 4 or even 8 hours - and yet it is a most significant basis for refinery control. Problems in measuring colour over a wide range of colour in whole raw sugars, as in the S.P.R.I. library of raw sugars, are far diminished, because:

1. Range of colour on washed raw is relatively narrow, compared to the library. Range will vary in depth according to refinery policy, that is, whether a single raw is melted at a time or whether raws are blended. Overwashing or underwashing of raw will, however, stand out when colours from individual centrifugal dumps are continuously monitored.
2. Depth of syrup coating on crystal will be reduced on washed raws and will contribute much less to error than in calibrations for whole raw sugars.

C. Soft sugars

The calibration was made on soft brown sugars with AGTRON™ values in the range of 19-61, and ICU in the range of 1176-10,049.

The validation for the calibration corresponding to AGTRON™ values is shown in Figure 8. The average of the AGTRON™ values is 45.6, and 43.7 of the prediction. SEP is 2.4. The validation was made almost 2 months after the calibration data were obtained. The validation show lower values from the ColourQ than the original AGTRON™ values, indicating that colours were darker at time of validation than at time of calibration. This darkening of soft sugars with time, is a well known phenomena in both light and dark soft sugars, with the colour increase generally correlated directly to moisture content of the soft sugar (9).

The validation for the ICU values is shown in Figure 9. The average of the ICU is 4292, and 3961 of the prediction. SEP is 929. To test the influence of the samples with high ICU values, all samples with ICU greater than 7000 were excluded from the validation set. This reduced validation set was measured with the same calibration. On this reduced set the average of the ICU is 3291, and 3121 of the prediction. SEP is 527.

One measurement system can have both AGTRON™ and ICUMSA calibrations installed, and can measure both values for simultaneous presentation.

From the comparison of laboratory and production line measurements on white sugars, the following prediction may be made: because measurements on soft brown sugars were made with the ColourQ 800 only in a laboratory system, and not on an on-line production system, it is to be expected that the precision of the results will be wider (not as good precision) than would be found for on-line measurement - that is, the laboratory measurement is "worst case" - an unusual situation, but understandable because of the difference in sample size.

Precision tests

The repeatability of the ColourQ 800 for both raw and soft brown sugars was tested by measuring a sample 10 times. For comparison a sample of raw sugar was tested 9 times by the conventional ICUMSA laboratory procedure. The results are shown in Table 3. The ICUMSA guideline (4) for repeatability of raw sugar is < 110 for sugars 500 to 2000 ICU and < 300 for sugars 2000 to 7000 ICU.

CONCLUSIONS

The automated, on-line Neltec system can give rapid, reliable and reproducible measurements for colour of white, raw and brown sugars. Comparison of the system with conventional laboratory measurements is summarised in Table 4, for time, accuracy, precision and degree of manpower.

1. For white sugars, the current study has confirmed that the Neltec system can be used to give accurate and precise colour measurement. It can provide an almost continuous analysis of wet sugar coming from centrifugals, thereby establishing a constant monitor for centrifugal performance and reduce costly recycling. It can monitor dry white sugar, out of driers or silo, thereby reducing out-of-specification problems and customer complaints and returns.
2. On raw sugars, this study has shown that the Neltec system can give colour measurement comparable to the ICUMSA measurement, under the worst conditions (widest range of raw sugar colour and quality). The speed of the Neltec system measurement allows an almost continuous analysis of raw sugar entering a refinery (or exiting a factory). The ability of the Neltec system to measure colour of wet sugar means that it can be used to monitor washed raw sugar colour in a refinery, monitoring centrifuge performance, and thereby reducing overwashing in affination with consequent energy savings.

It can equally well be applied to measure raw sugar colour in a raw sugar factory (or blending station where VHP raws are coated), offering continuous control of raw sugar colour, with similar benefits for centrifugal control. Problems in centrifugal operation can be identified in a few seconds. Wash levels can be minimised, and amounts of sugar lost to A or B molasses can be reduced. For raw sugar factories producing a high grade edible speciality product, the indication that the Neltec instrument correlates better with visible colour than does the ICUMSA measurement may be a useful property.

3. For soft sugars, the Neltec instrument offers the opportunity for rapid measurement of each batch of sugar boiled, or a continuous measurement of colour in blended (coated) sugars. The colour can be read

simultaneously in both ICUMSA units and the AGTRON™ units popular in North America. There is space for 5 calibrations (or simultaneous readings) so a calibration for each of the various types of AGTRON™ instruments could, if wished, be used to satisfy requirements of different customers. In summary, the Neltec system can be used on-line in the raw sugar side and the final product side (white or brown) of a refinery to provide rapid, reliable measurement of colour.

ACKNOWLEDGMENTS

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Table 1. Samples for calibration and validation.

	CALIBRATION			VALIDATION		
	# samples	Mean	Range	# samples	Mean	Range
Raw sugar (ICUMSA, buffered)	89	4744	238-15457	14	4634	2299-6145
Refined cane sugar (ICUMSA, buffered)	50	41.1	7.9-73.6	16	39	7.4-64.0
Soft sugar (AGTRON™)	55	44.8	19-61	18	45.5	19-61
Beet white sugar (ICUMSA, buffered)	234	34	25.0-104.8	n/a	n/a	n/a

Table 2. White cane sugar from refinery.

Buffered		
Refinery lab	Neltec	Neltec Rep.
7.4	7.9	7.7
15.5	12.0	13.5
29.1	26.3	29.9
24.5	21.8	25.0
34.3	29.0	33.1
34.4	35.1	38.1
45.8	43.4	48.0
45.9	44.9	49.5
53.0	51.3	53.2
56.6	53.7	53.0
48.8	49.5	49.0
57.2	50.3	50.8
19.5	22.6	23.9
30.8	34.5	33.9
57.1	55.3	55.0
64.0	62.7	64.8
SEP	3.1	2.8

Table 3. Precision test - statistical analysis of 10 replicates.

Repeats	RAWS				SOFTS	
	ICUMSA by Neltec	abs residual	ICUMSA by Lab	abs residual	AGTRON™ by Neltec	abs residual
1	3239	31	3073	155	24.4	0.93
2	3205	65	3013	95	22.4	1.07
3	3305	35	2941	23	23.4	0.07
4	3289	19	3038	120	22.8	0.67
5	3305	35	2962	44	23.8	0.33
6	3317	47	2873	45	23.7	0.23
7	3233	37	2775	143	22.9	0.57
8	3250	20	2785	133	24.0	0.53
9	3283	13	2805	113	23.6	0.13
10	3274	4			23.7	0.23
mean	3270	31	2918	97	23.5	0.28
Std. dev.	37	18	113	48	0.6	0.34

Table 4. Comparison of standard colour measurement with Neltec system measurements.

		Standard (laboratory)	Neltec
Time	ICUMSA colour	15-20 min	5 sec
	RBU	6-10 min	5 sec
	AGTRON™	2-4 min	5 sec
Accuracy	White	±2 ICU	Similar but larger than method used for calibration
	Raw	±100 ICU	
Precision	White	3	2.9
	Raw	113 (100-300)	37
	Soft	1.0	0.6
Labour level		Intensive, requires training	Very low

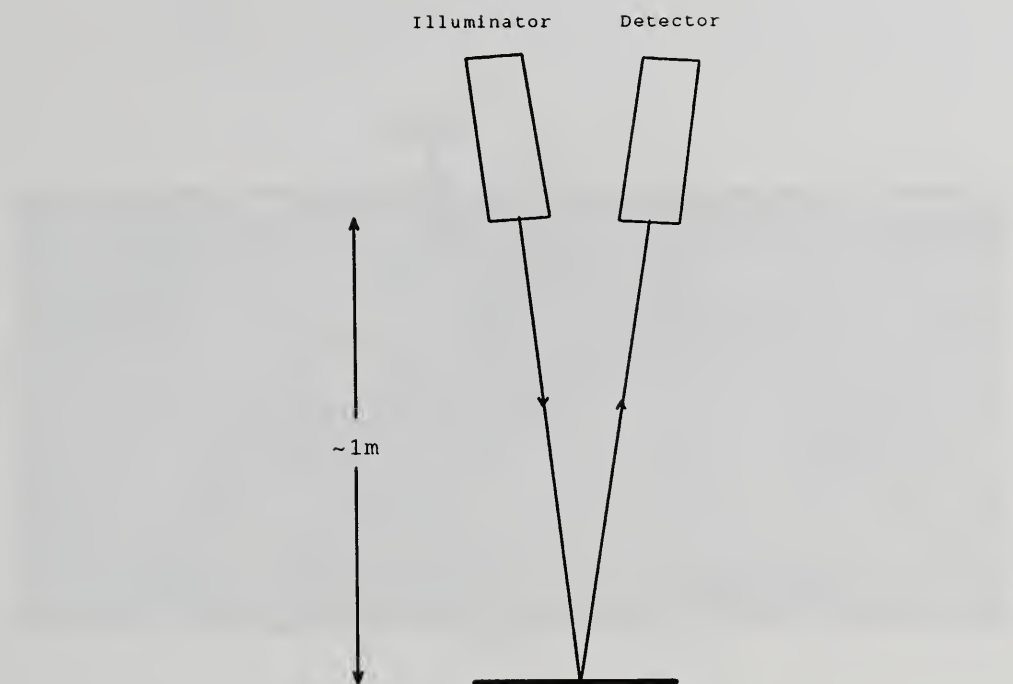


Figure 1. Position of illuminator and detector over sugar.

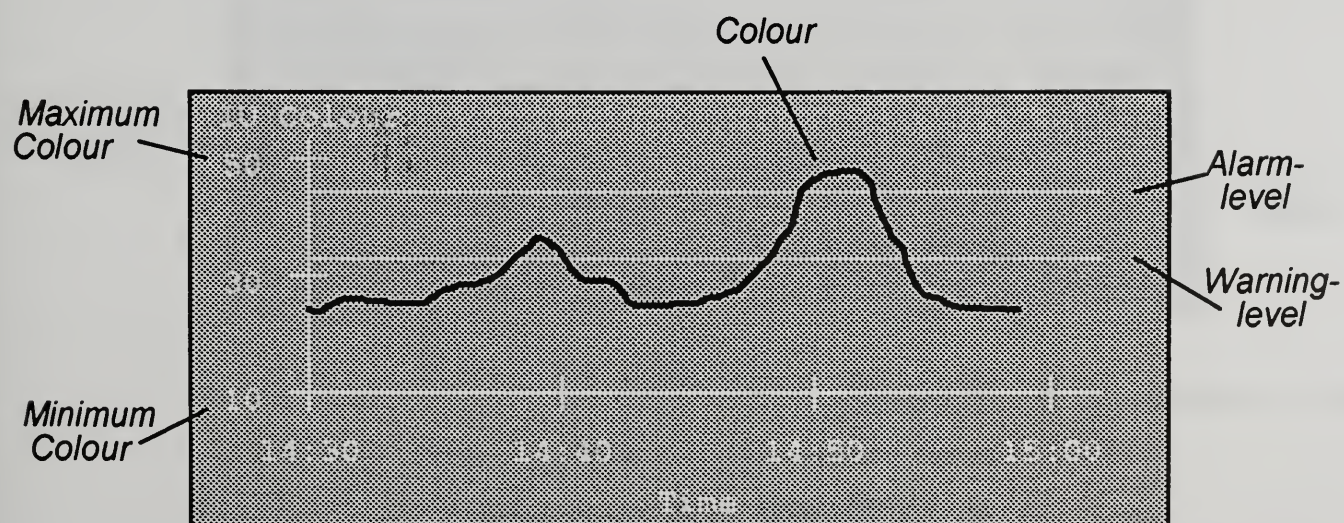


Figure 2. Short term graphics.

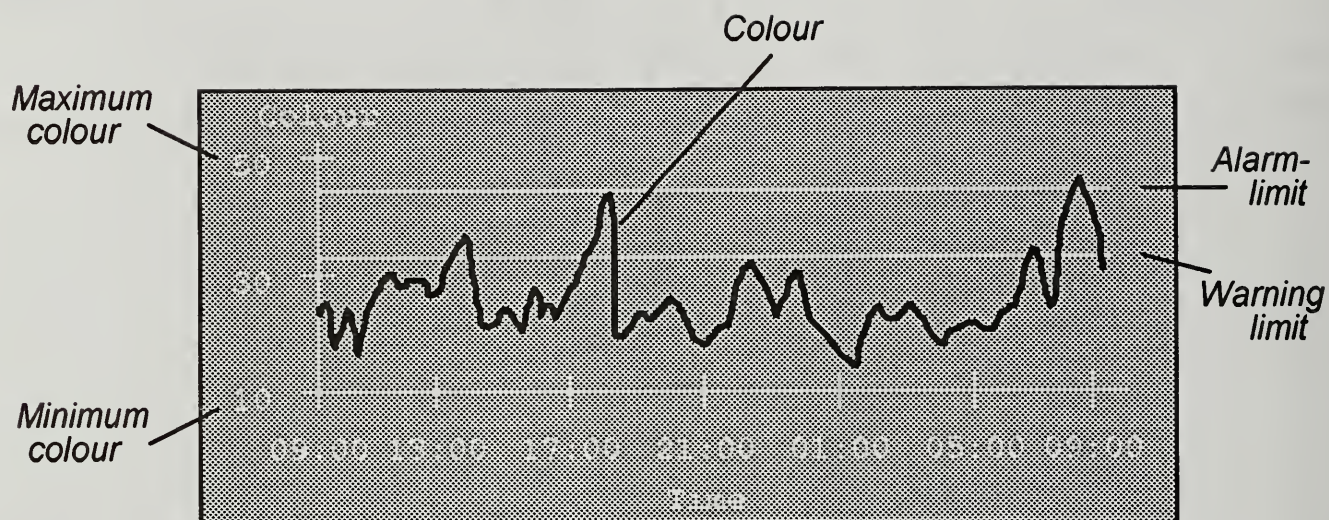


Figure 3. Long term graphics.

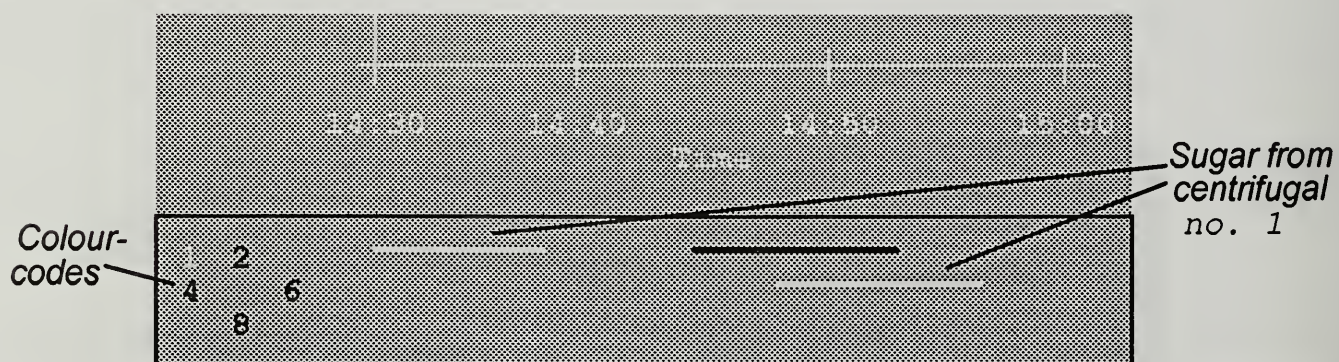


Figure 4. Identification of centrifugals.

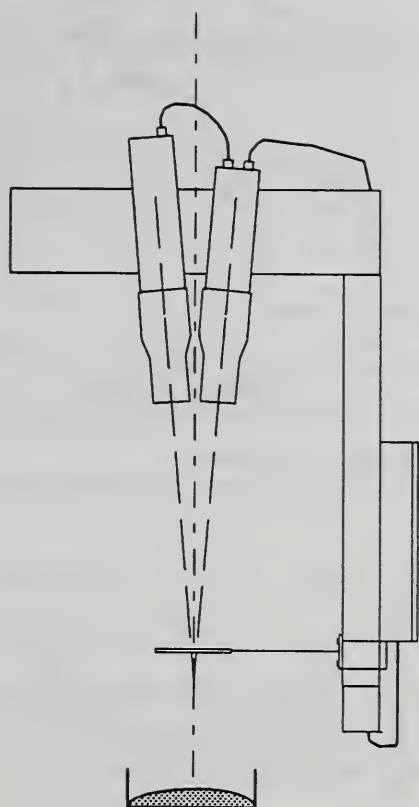


Figure 5. Measurement of dry sugar in sample tray.

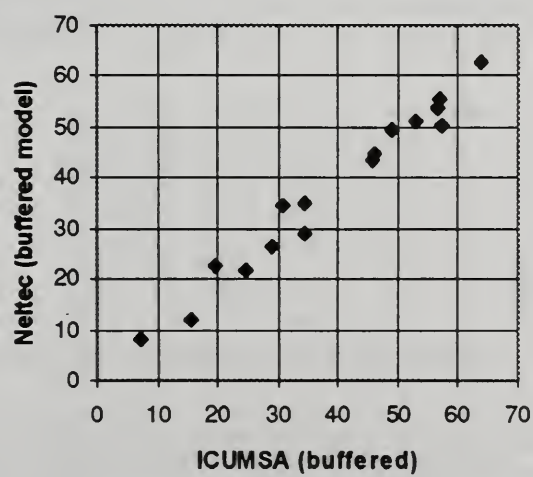


Figure 6. Validation of model for white cane sugar.

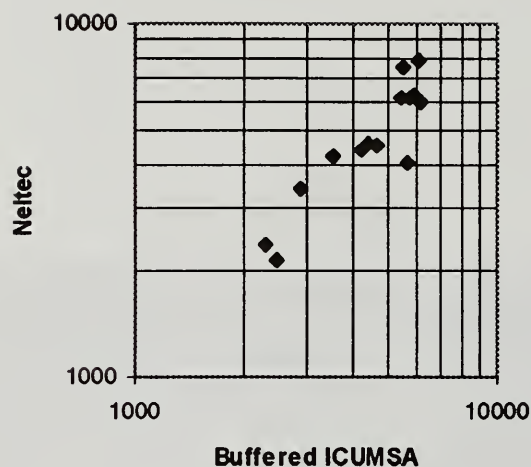


Figure 7. Validation of model for raw sugars, buffered.

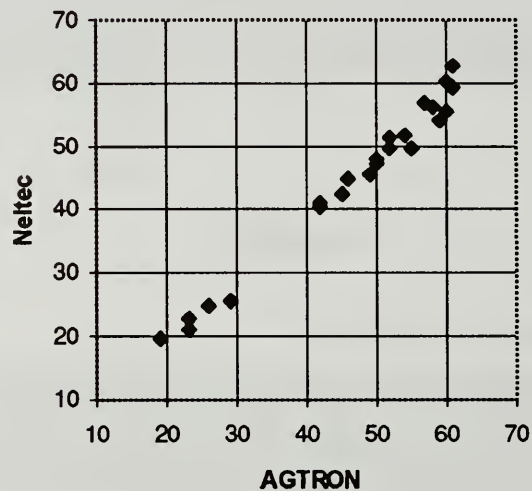


Figure 8. Soft sugars, AGTRON validation.

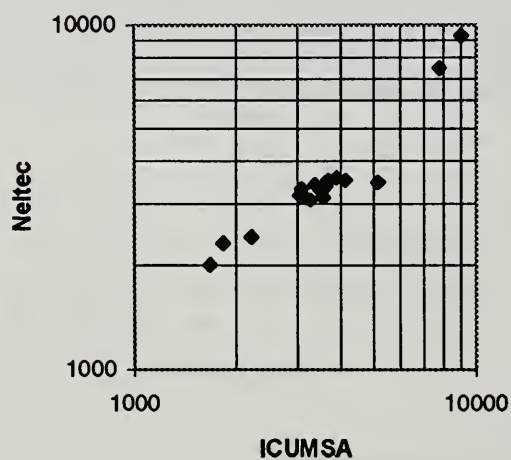


Figure 9. Soft brown sugar, ICUMSA validation.

POSTER

CONTROL OF THERMOPHILIC SPORE-FORMING BACTERIA IN SUGAR PROCESSING

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ABSTRACT

Thermophilic spore-forming bacteria are of concern to sugar processing technologists. Their resistance to thermal treatments, and on the other side, the needs of customers, specially the "Canners", demand vigilance.

In this work we examine the relationship between each process unit operation and these microorganisms.

INTRODUCTION

Thermophilic spore-forming bacteria may enter the refinery in raw sugar, and can colonize the plant, maintaining a presence by means of vegetation, sporulation and revegetation cycles. They live as spores in adverse conditions (for example heat, desiccation), and as vegetative cells in more favorable environments. [1]

According to Gene Snook et al [1] the predominant carbohydrate fermenters identified (by them) are thermophilic spore-forming, characterized as *Bacillus* species. Although they have an important role in losses of sucrose, we did not quantify these losses. Our aim was to find a way to decrease the bacterial counts in sugar, in order to improve its microbiological quality.

With the work here described, we determined the distribution and the intensity of the microbial populations, through the unit operations main line, in the refinery. Also we tried to examine the self-defense capacity of Process for this problem.

EXPERIMENTAL**SAMPLING STRATEGY**

Samples were collected immediately before and after unit operations.

For each experiment, the set of samples were obtained in such a way that the portion of material sampled along the points under test, was the same. In other words, the interval between two samples from two points, matched the time needed for the sugar stream to pass through the part between these points.

ASEPTIC CONDITIONS

Almost all points of sampling used tube and tap. After blazing (sterilizing) the tube, the tap was turned, enough juice was rejected in order to get a representative sample of the stream, and the sample was collected in a sterilized plastic bag.

For other points without a tube and tap, and for sugars, sterilized metallic containers with a catching part were used. Immediately after the collection, these containers were covered with sterilized plastic bags.

ANALYSIS

Once arriving at the laboratory, samples were cooled rapidly with running water and, as soon as possible, were analyzed for refractometric solids percent, pH (of liquids) and thermophilic spore-forming organisms.

MATERIALS

Culture medium: Dextrose Tryptone Agar – Oxoid CM75 with 1g/L of *di*-Ammonium hydrogen phosphate in order to ensure the culture pH – 6.9.

MICROBIOLOGICAL ASSAY (Based on ICUMSA method GS2/3 – 49 - 1994) [2]

Sample amount containing 10g of dry sugar matter is dissolved in 50ml sterilized water.

The solution is heated quickly in a boiling water bath and maintained at 100°C for exactly 5 minutes.

The solution is cooled quickly with running cold water until circa 50°C.

Volume is made up to 50ml if necessary.

25 mL of solution are mixed carefully with 75 mL of already prepared medium. This portion of medium contains the dry components prescribed for 100 ml.

Immediately poured into 6 Petri dishes.

Incubation : 55°C for 48 hours

RESULTS

Expression of results: colonies forming units per 10g of dry sugar matter (CFU/10g DSM).

“Flat-sour” thermophilic spore-forming: count all colonies surrounded by a yellow zone (in contrast with purple medium) showed by all petri dishes and multiply by 2.

Total thermophilic spore-forming : count all colonies showed by all petri dishes and multiply by 2.

RESULTS AND DISCUSSION

The experiment was repeated several times over three months.

For the bacteria under study, in all sets of samples, charts having the enumeration of colonies forming units per 10g of dry sugar matter (CFU/10g DSM), show one typical profile - figures 1 and 2. Beside this, we found one exception several times, another profile – figures 3 and 4.

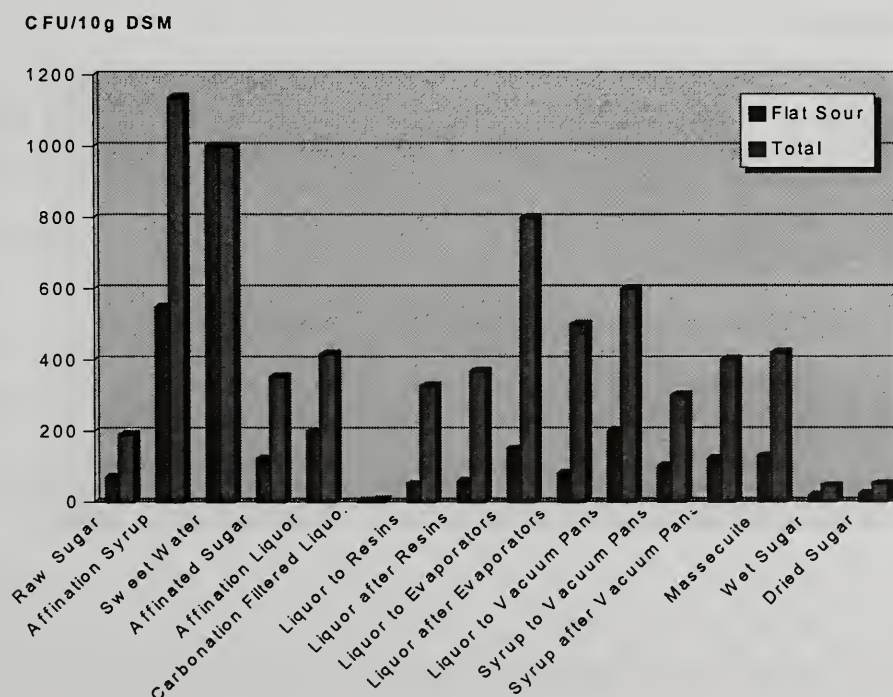


Figure 1. Typical profile for CFU/10g DSM of Thermophilic spores, “flat-sour” and total, in each product of process.

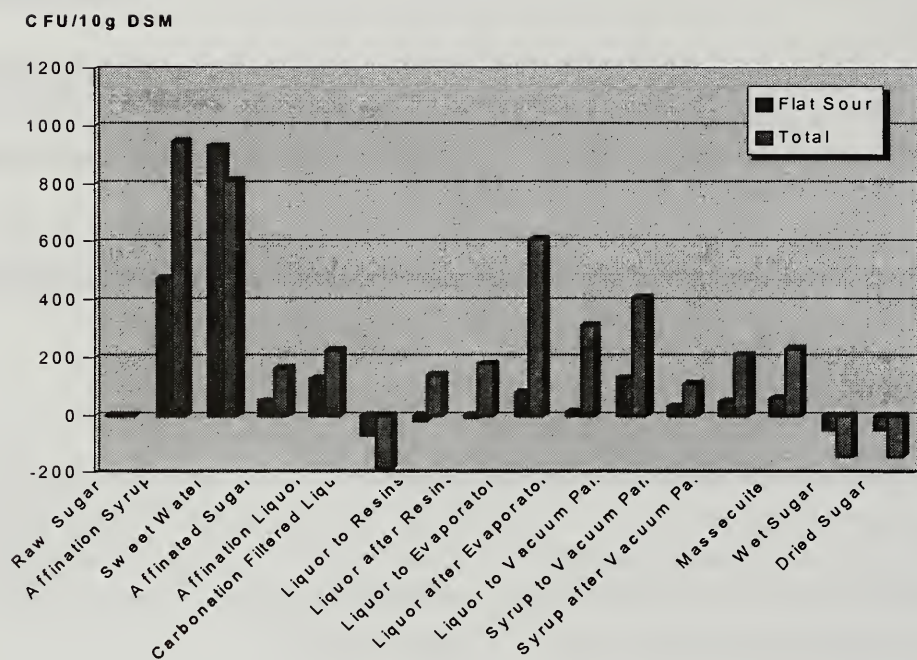


Figure 2. Differences of thermophilic spores content, between each product of process and the raw sugar from which they derived, under a typical situation.

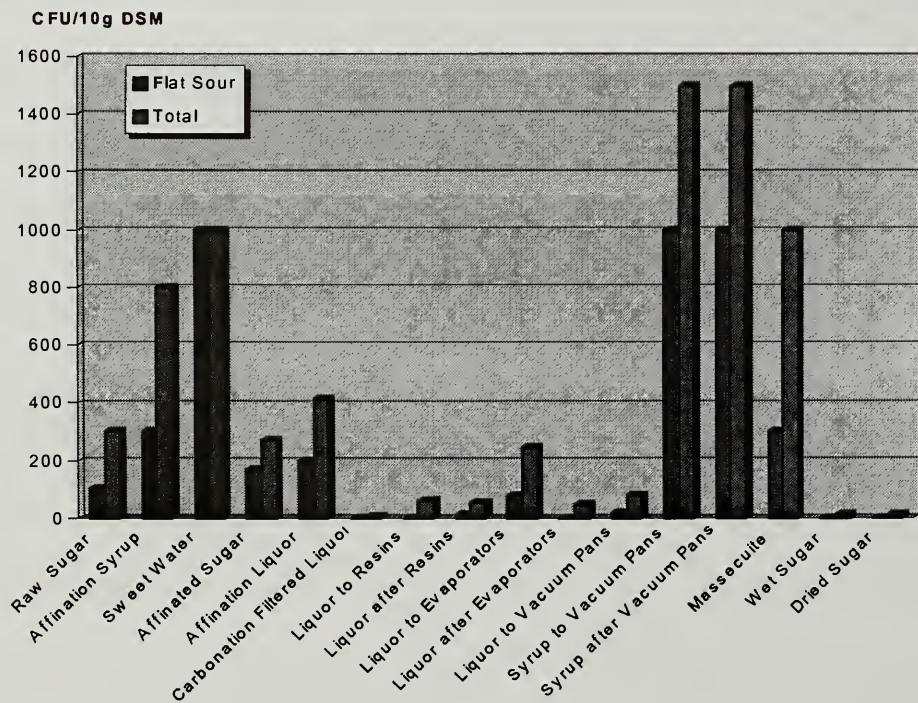


Figure 3. The exceptional profile found for CFU/10gDSM of Thermophilic spores, “flat-sour” and total, in each product of process.

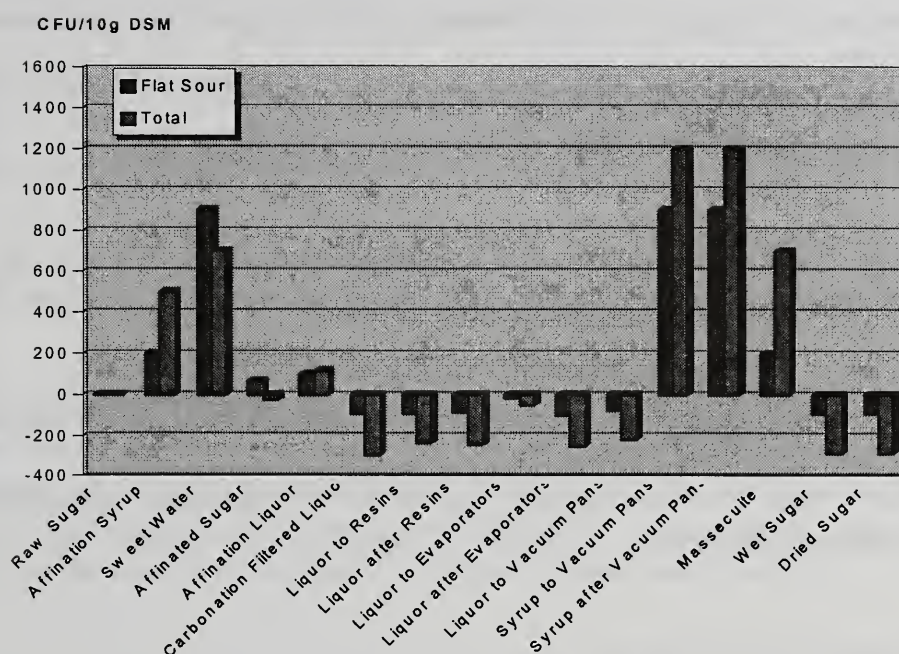


Figure 4. Differences of thermophilic spores content, between each product of process and the raw sugar from which they derived, when occur the exception of typical situation.

Charts show:

Carbonated liquor after filtration had very few or no thermophilic spore-formers. This result was been obtained without exceptions.

On a dry matter basis, affination syrup and sweet waters, had much more bacteria belonging to this group than raw sugar.

Figures 3 and 4: Syrups to and from vacuum pans, have an increased content, of Thermophilic spores, if we compare with raw sugar and with the same products analysed at other days.

These bacteria multiply in each liquor during the delay in storage tanks.

CONCLUSIONS

Carbonatation removes the thermophilic spore-formers from process streams, with very good efficiency.

Due to the metabolic characteristics of thermophilic spore-formers and conditions of temperature and pH, storage tanks are critical points.

An external contamination of syrups in crystallization, as well as massecuite, can occur, possibly coming from vacuum condensers.

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The authors wish to thank their colleagues.

POSTER

THE OCCURRENCE AND ISOMERISATION OF ACONITIC ACID IN FACTORY PROCESSING

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INTRODUCTION

The SMRI has been involved in a multi disciplinary research program into the causes and possible prevention of evaporator scale (7). A chromatographic method was used to monitor levels of organic acid species present in both scale and factory streams (3). During this work it became apparent that some of the aconitic acid (the major non- nitrogenous acid found in cane process streams) isomerised from the *trans* to the *cis* form in the factory. The ratio of *cis* to *trans* was found to increase from the front to back end of the factory. This study considers some of the factors influencing this isomerisation

EXPERIMENTAL

The experimental procedures, methods and equations used in this study have been reported previously (3,4).

DISCUSSION

Typical *cis/trans* ratios found in South African factory streams are given in Table 1. Values recorded are the average found over a three year period. The greatest increase is found across the evaporator station and between syrup and molasses. Average aconitate acid levels vary between 5000 and 8000 ppm/Bx depending on time of season and geographic area.

The structures of the two isomers (*cis* and *trans*) are shown in Figure 1 with a typical isomerisation concentration profile shown in Figure 2. The isomerisation follows a pseudo first order rate equation given by the following equations:

$$\ln \left[\frac{[trans] - [trans^{eq}]}{[trans^0] - [trans^{eq}]} \right] = -(k_1 + k_{-1})t \qquad \ln \left[\frac{[cis^{eq}]}{[cis^{eq}] - [cis]} \right] = (k_1 + k_{-1})t$$

Once the isomerisation has reached equilibrium, the equilibrium ratio (K) is given by:

$$\frac{k_1}{k_{-1}} = \frac{[cis^{eq}]}{[trans^{eq}]} = K$$

The effect of pH, temperature and ionic strength on the isomerisation rate can be quantified using these equations.

pH

The effect of pH at 97°C is to increase the rate of both forward and reverse rate as the pH decreases (figures 3a, b). The equilibrium ratio also increases with the decrease in pH (Table 2); however the ratio would appear to be independent of temperature.

Temperature

The Arrhenius equation is used to express the rate of reaction as a function of temperature:

$$k = A \exp\left(-\frac{E_a}{RT}\right)$$

where A = pre-exponential factor E_a = activation energy
 R = gas constant T = temperature (°K)

Figure 4 shows the dependence of the forward rate upon the temperature expressed in the Arrhenius equation form. It can be seen that the rate increases as the temperature increases at a rate of between 2 and 3 times per 10°K temperature rise. A similar graph can be developed for the reverse rate which shows a similar trend. The effect of temperature on the rate is more pronounced at pH 7 than at pH 5 (~1.4 times faster).

Ionic strength and cation composition

Plotting the square root of the ionic strength against the log of the forward rate shows the rate increasing with an increase in ionic strength (figure 5). This has possible implications at the back-end of the factory where sucrose is being removed and ionic concentrations increase. To a lesser extent the same can be applied to the evaporator station. A similar graph can be constructed for the reverse rate.

The effect of cations can be measured by replacing the sodium cation with either potassium, calcium or magnesium. When measured at 90°C and pH 5, there appears to be little difference in the isomerisation rate when sodium or potassium are present as cations (figure 6). However magnesium and calcium at 1:1 and 1:30 aconitate:cation ratio inhibit the rates slightly with calcium having the greater effect. These aconitate:cation ratios are typical of factory processing streams. No difference appears in the equilibrium constant K, implying that only the rate of isomerisation is slower.

Breakdown products

Chromatographic analysis (4) (ion-exclusion and reverse phase chromatography) has shown the presence of a number of breakdown products appearing as isomerisation reaches equilibration (figure 7). The itaconic acid formed by decarboxylation of the *trans* isomer is the major compound formed at pH 5 when aconitic acid is heated by itself. The presence of other cations (calcium, magnesium) and buffers (acetate and phosphate) inhibits formation of itaconic acid but enhances the formation of other peaks (especially peaks 3 and 7). Analysis on a reverse phase system has also shown oxalate formation. This study is under further investigation.

Evaporator studies

Simultaneous studies of the aconitate ratios across the evaporator station and in associated scale show that the *cis/trans* ratio increases across each effect. The greatest increase is found in the 1st and 2nd effects (where the temperatures are highest) (figure 8a). The isomer ratio in the juice and scale from each effect is similar, indicating that either both isomers precipitate at the same rate (unlikely (5)) or that most of the aconitate in scale comes from inclusion of juice. Isolated incidents of **precipitated** aconitate have been recorded for two inland mills (up to 33% of the scale). These mills show consistently higher aconitate in their scale (figure 8b) and lower *cis/trans* ratios than the other mills in the industry. It has been suggested that the mineral composition of the soils in the region may be responsible for this phenomenon (6).

Process possibilities

The measurement of organic acids, and in particular aconitic acid, has allowed the evaluation of possible process changes on juices and scales.

Preliminary studies of ozone for decolourising at high dosage rates (1000 to 3000 ppm on solution) showed that aconitic acid formed an ozonide which hydrolysed to other acids including malic, glycolic and oxalic (3). The *cis/trans* ratio did not change with increasing ozone dosage, indicating that both isomers probably underwent ozonolysis (figure 9a,c).

The use of sulphur dioxide as an antiscalent (7) shows that the ratio of oxalic, glycolic and malic acids did not change from the control syrup with increasing SO₂ addition (figure 9b). However the quantity of aconitic acid decreased, due to formation of a sulphate. This would appear to be a reaction of the *trans* isomer due to the increase in the *cis/trans* ratio with increasing sulphur dioxide. The aconitate scale formed from this juice was reduced by about 60%.

Refining

Analysis of refinery streams has shown that aconitic acid is removed during carbonation and subsequent filtration. Figure 10a plots the average aconitate levels from the melt to the fine liquor stage. The levels of

aconitate drop approximately 90% between melt and clear liquor. The *cis/trans* ratio at the melt, limed liquor and fine liquor stages are similar but the clear liquor ratio was consistently higher. Both isomers appear to precipitate with the calcium and to be removed in the filtration stage (decrease in concentration).

The increase in the *cis/trans* ratio from melt to clear liquor shows that the *trans* aconitic acid is removed by reaction at the high pH to form other compounds. Evidence for this reaction is the increase in glycolic acid formation across these stages (figure 10b). These observations agree with the work of Otake et al (8) who showed that total aconitic acid decreased across carbonatation and glycolic acid increased. As the pH drops across the sulphitation stage, isomerisation of the *cis* and *trans* isomers will occur to bring back equilibration of the isomers.

CONCLUSIONS

Aconitic acid isomerisations are affected primarily by pH and temperature. Lower pH and higher temperatures lead to faster rates of isomerisation. Although ionic strength is not as important a factor, increasing ionic strength also accelerates the rate of isomerisation. The presence of calcium and magnesium marginally inhibits the isomerisation. However, the presence of these divalent cations inhibits decarboxylation of *trans* aconitic acid to itaconic acid but enhances the formation of other breakdown compounds.

The greatest isomerisation of the *trans* acid occurs in the evaporators, particularly in the 1st and 2nd effects where the temperature is highest. Further isomerisation takes place in the pans as sucrose is removed and ionic strength of the masscutes increases.

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Table 1. Typical aconitate ratios in factory streams.

	<i>cis/trans</i> ratio	Range
Fresh cane	0.03	0.01 - 0.04
Mixed Juice	0.05	0.03 - 0.08
Clear Juice	0.07	0.05 - 0.11
Syrup	0.15	0.08 - 0.21
Molasses	0.27	0.19 - 0.41
A-sugar	0.19	0.15 - 0.21

Table 2. Effect of pH on the equilibrium constant K.

Temp (°C)	pH 4	pH 5	pH 6	pH 7	pH 8
70		0.372	0.235	0.129	
80		0.410	0.260	0.133	
90	0.591	0.413	0.271	0.147	
97		0.412	0.275	0.144	0.125

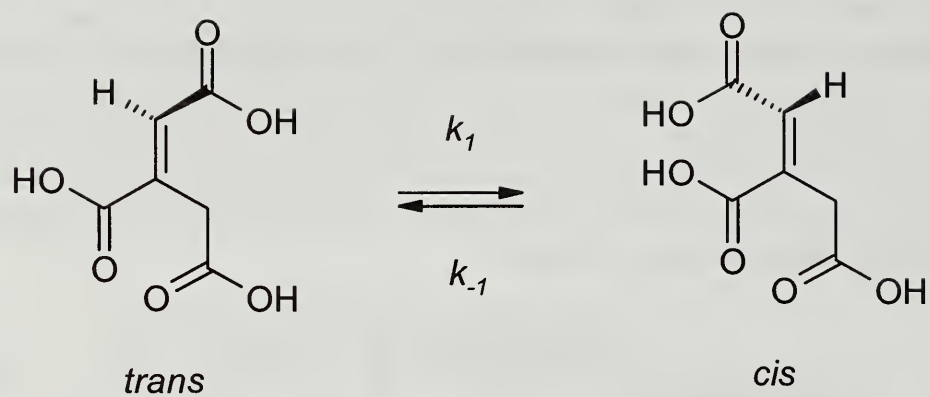


Figure 1. Structures of *cis* and *trans* aconitic acid.

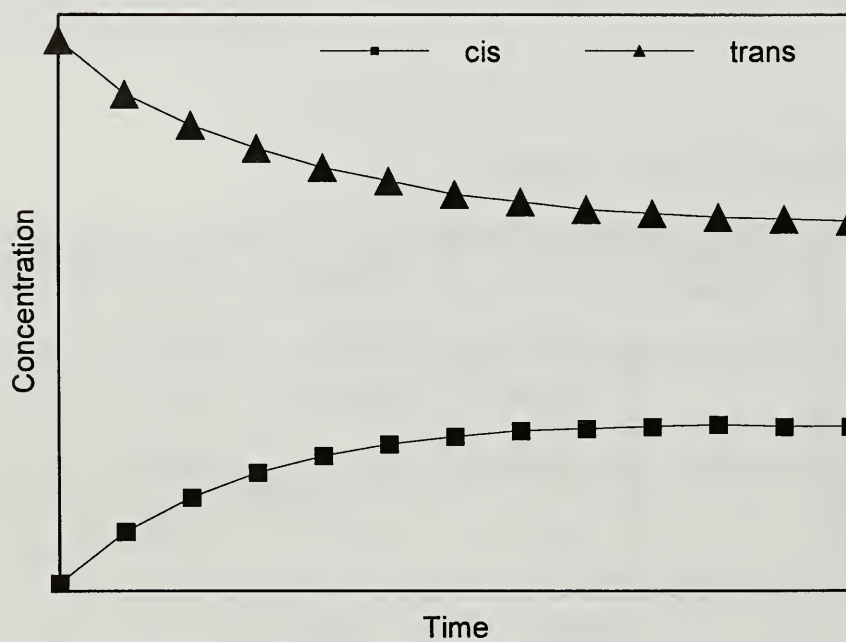


Figure 2. Typical isomerisation concentration profile.

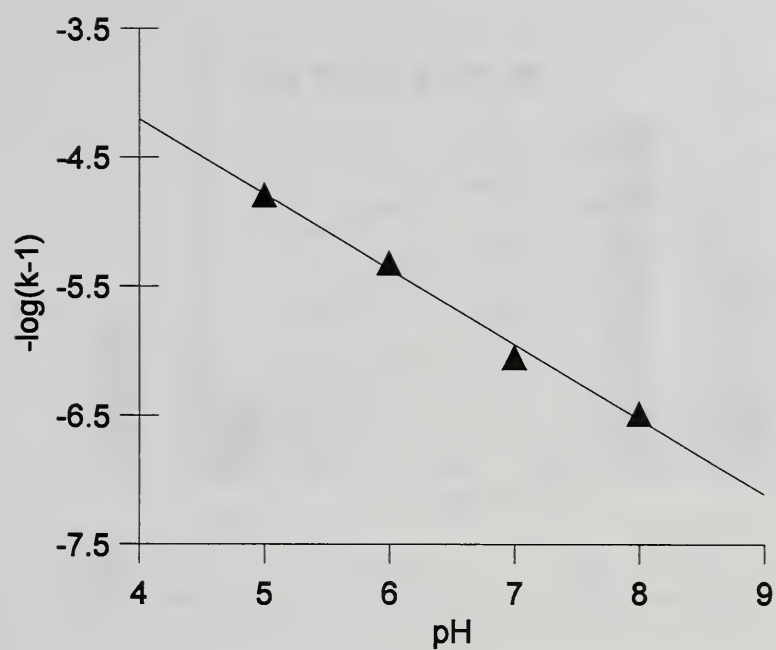


Figure 3a. Effect of pH on the forward isomerisation rate at 97°C.

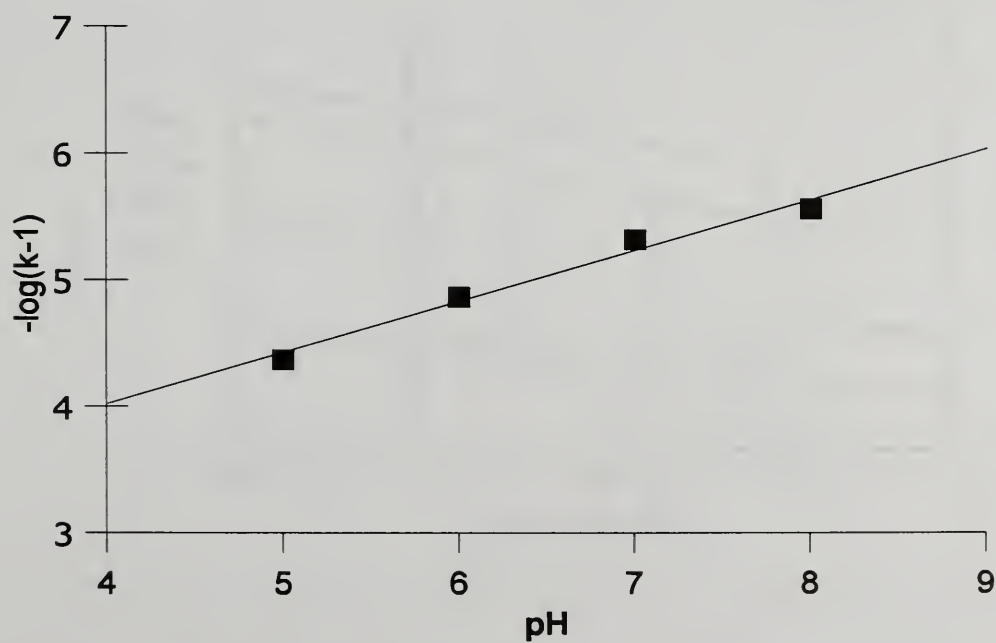


Figure 3b. Effect of pH on the reverse isomerisation rate at 97°C.

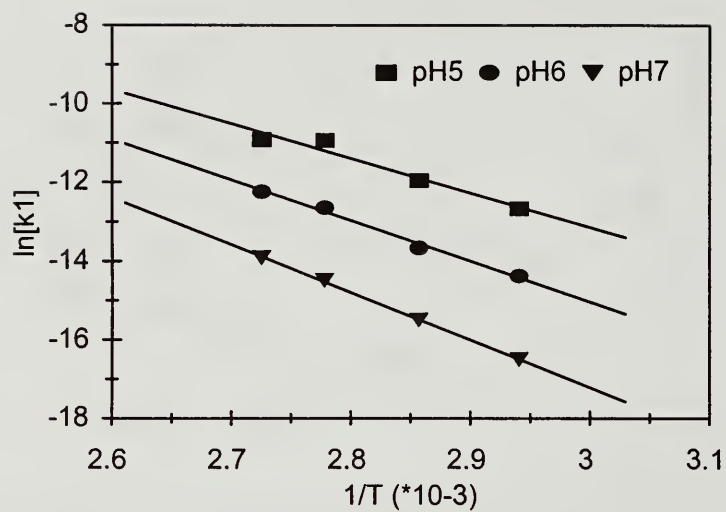


Figure 4. Effect of temperature on the forward isomerisation rate.

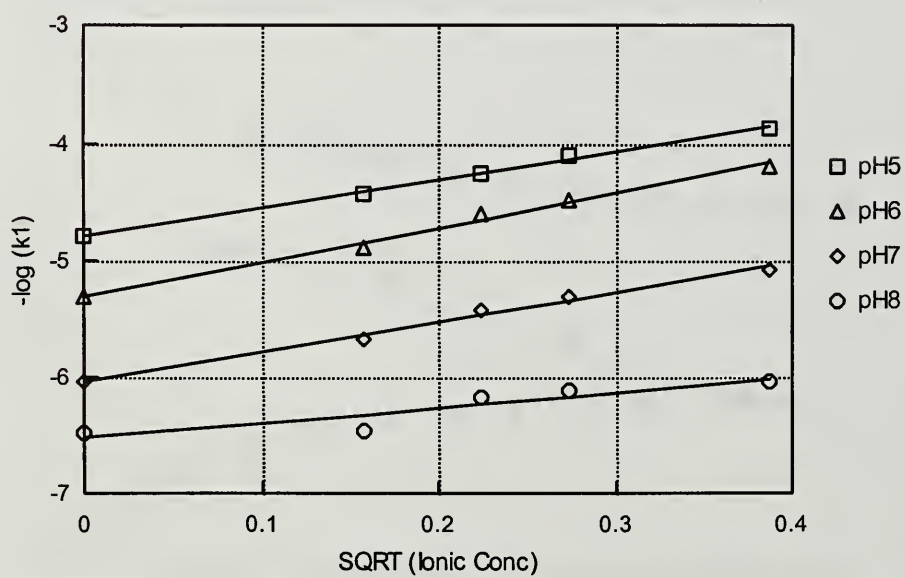


Figure 5. Effect of ionic concentration on the forward isomerisation rate.

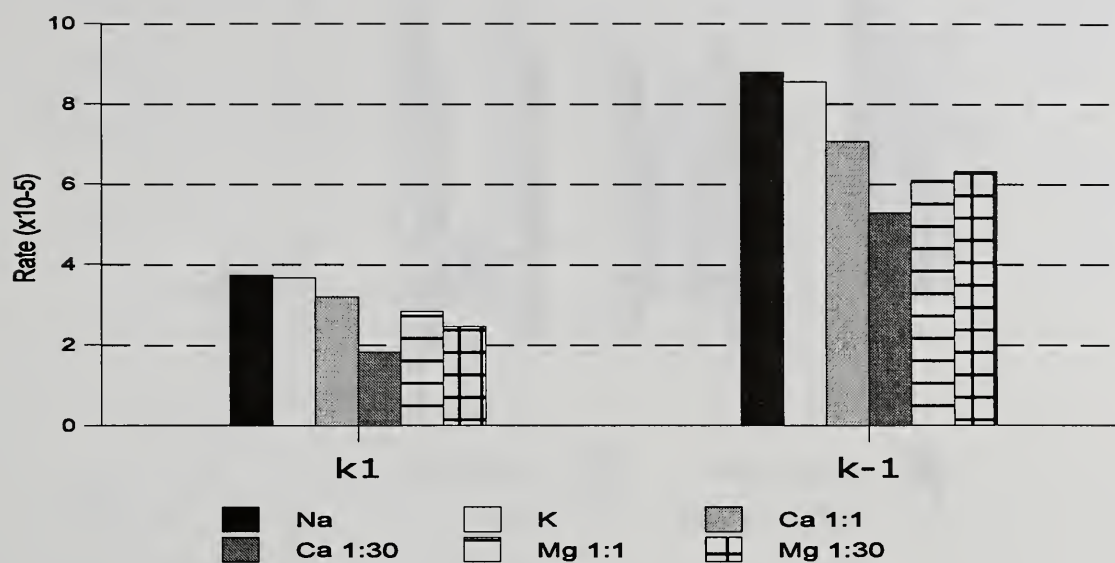


Figure 6. Effect of cations on isomerisation rate.

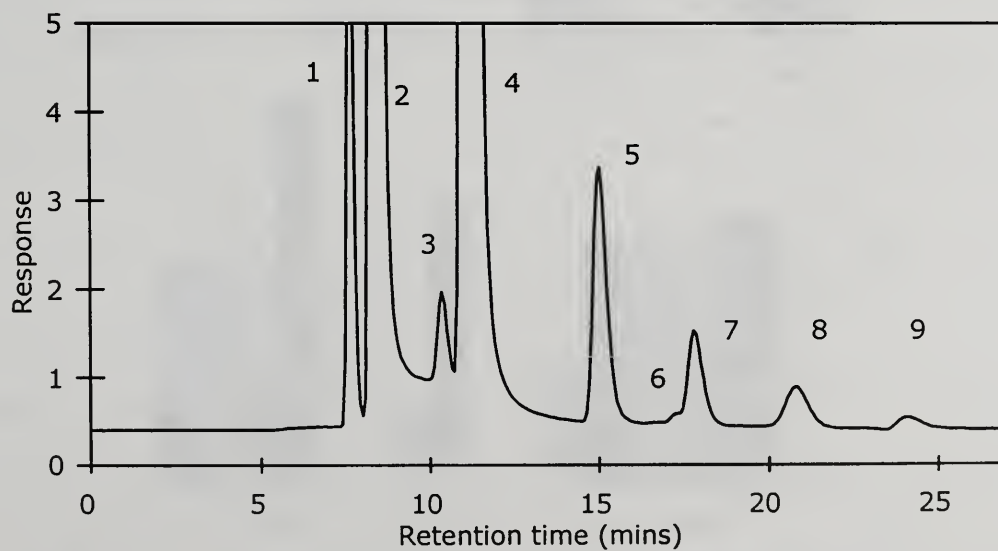


Figure 7. Ion exclusion chromatogram of an equilibrated isomerisation mixture. 1=solvent, 2=*cis*, 4=*trans*, 5=itaconic acid, 3,6,7,8,9-unknown.

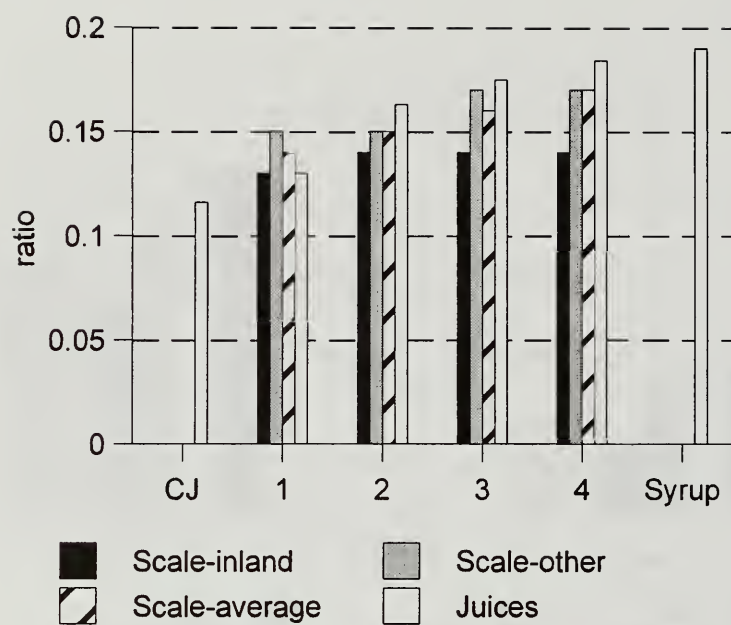


Figure 8a. *cis/trans* ratio in juices and scale from inland and other mills.

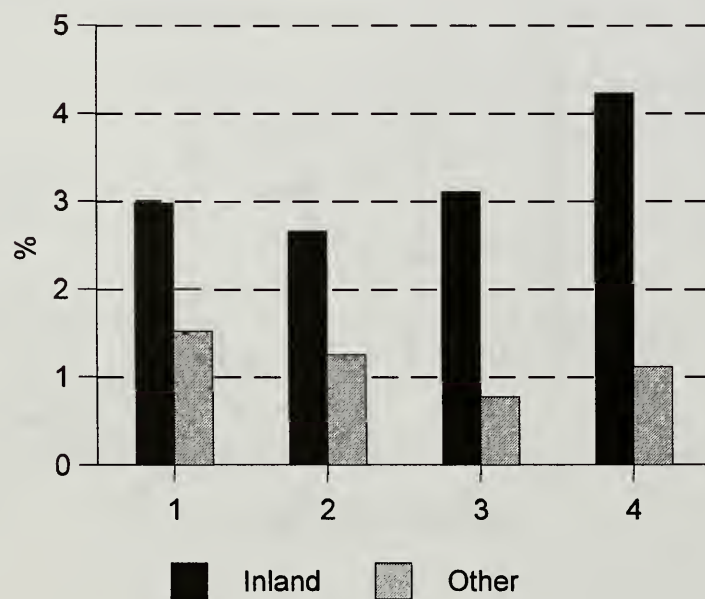


Figure 8b. Total aconitine (5) in scale from inland and other mills.

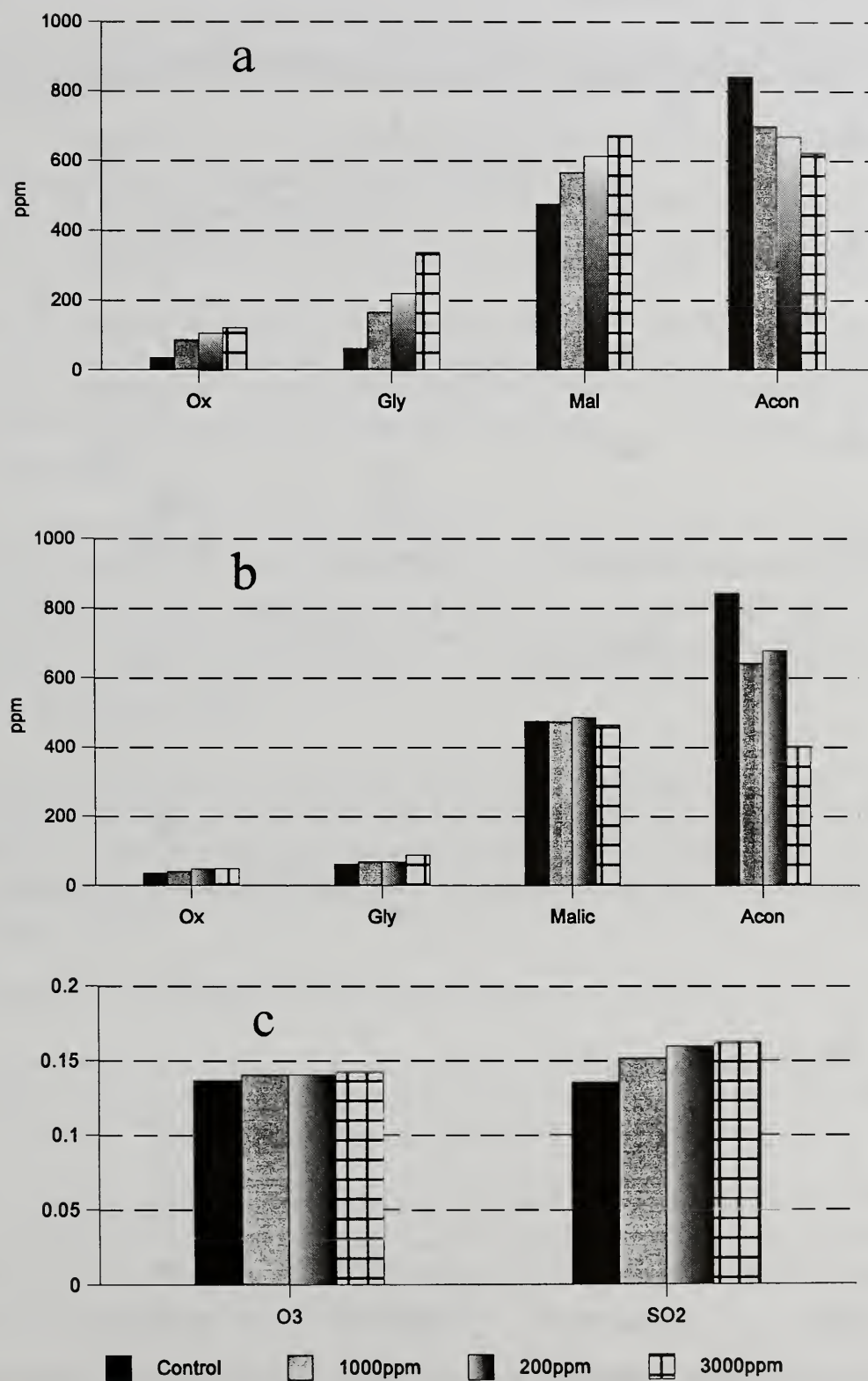


Figure 9. Organic acid profiles for (a) ozone treatment, (b) sulphur dioxide treatment and (c) *cis/trans* ratio.

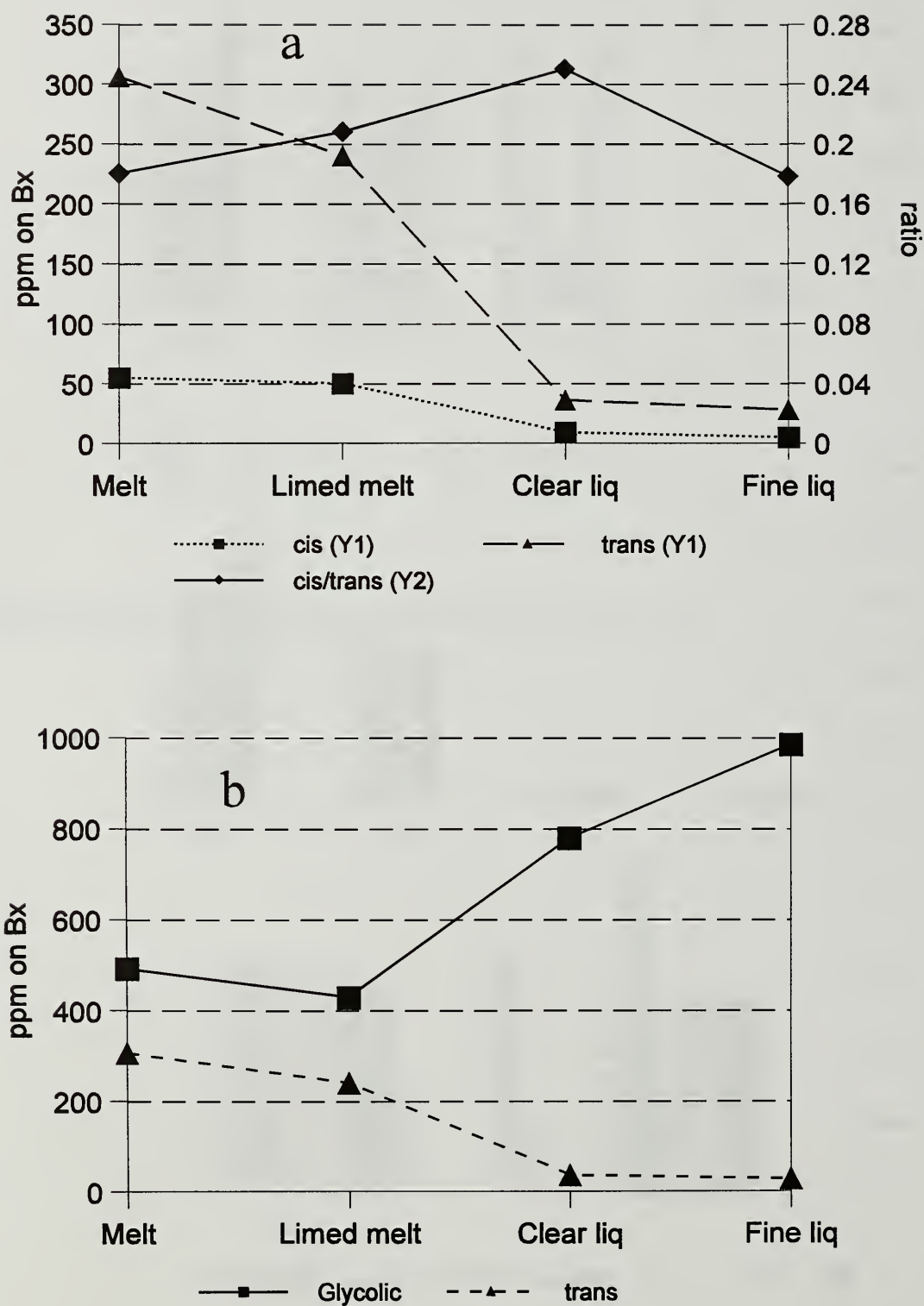


Figure 10. Aconitate levels and cis/trans ratio (a) and glycolic acid levels (b) across the refinery.

POSTER

PRELIMINARY STUDY OF SOME SUGARCANE VARIETIES AGAINST "ROUNDUP™" APPLICATION

Lourdes Quesada, Ulisses Osegueda and Otto Kopper

Consultores Asociados Lourdes Quesada, S.A.

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For five consecutive weeks, seven different Hawaiian sugarcane varieties, of 13 months average age, were tested in Costa Rica for their response to the chemical ripener, Roundup™. The field chosen for the study was divided into two sections, one to be treated with Roundup™ and the other without, to act as the control. Sampling began the fourth week after Roundup™ application. The altitude of the planting was another variable in this study.

The purpose of this study was to find out if there was a difference in the cane composition between treated and untreated cane that could affect yield in the factory. For several years, the factory had been experiencing problems with high viscosity and poor crystal recovery. The parameters chosen for analysis included polarization, reducing sugars by high performance liquid chromatography (HPLC), Brix, filter cake, mud quantity, sucrose by HPLC, dextran and starch. Cooperators from the Department of Agriculture helped to choose the field sampling sites.

The cane sample was taken from a square meter plot, and was divided into two equal parts. The sample also contained the soil and trash elements included in harvesting. These were prepared to obtain juice and filter cake. Methods of analyses were recommended by Dr. Margaret Clarke and included SPRI and ICUMSA methods. Sugar analysis was done by HPLC in isocratic mode, with a constant temperature oven and an anion exchange column.

It is envisioned that this study will take at least five years to complete.

The cane in this study was subject to a higher altitude and different weather conditions than those in the rest of the country. Because of the altitude, there is a lot of rain during the harvest, with an average ambient temperature of 25°C. The varieties ranged in age from 13 to 18 months.

The results of the study showed that starch content in the canes was the most significant variable. Varieties could be classified as low, medium and high starch varieties. In addition, starch content was a consequence of the maturity of the cane, with starch decreasing as cane matured. High starch in the cane juice coming into the factory was the cause of the high viscosity.

It is recommended that the harvest program be based on the analytical content of the starch in the juice. Neither Brix nor pol, the usual parameters tested, will indicate whether starch is present. Cane to which Roundup™ had been applied had a different starch content than the control canes. Analysis of the starch can indicate an optimum time for cane harvest.

S.P.R.I.

Yield was increased in cane treated with Roundup™, but fiber content decreased, probably because the cane did not grow as tall or as thick as the control cane. Since bagasse is used as a source of energy to operate the mill, this decrease in fiber could pose a problem. This will have to be confirmed by further study.

Acknowledgment: Thanks to Ingenio Hacienda Juan Viñas, Ing. Jorge Salazar, Agriculture Department and the personnel at LAICA.

POSTER

NEAR INFRARED (NIR) ROUTINE ANALYSIS OF SUGARCANE JUICES

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ABSTRACT

A near infrared NIRSystems Beverage Analyzer was utilized at the Juice Quality Laboratory, Ardoyne Farm, Houma, Louisiana, during the 1995-96 harvest season for the analyses of sugarcane juice samples of experimental clones from the U.S. Department of Agriculture, Agricultural Research Service variety development program. The Beverage Analyzer instrument has two fixed probes so that no sample preparation was necessary. Results from 500 comparative tests for Brix and 350 tests for pol using the Beverage Analyzer and conventional methods compared favorably. The correlation coefficients for both parameters exceeded 0.97 and precision was good. This NIR method provides fast and accurate results without the use of any chemicals or clarification agents. It is therefore an environmentally friendly method.

INTRODUCTION

NIR of sugarcane juice for pol and Brix is well established (2) as a proven analytical technique, and is in use as analysis for cane payment by factories in the State of Sao Paulo, Brazil. The original method of presentation of the juice sample required further development to increase speed of analysis, to permit the analysis of a large number of samples in a short time. Initial trials used a quartz cuvette, usually 1 mm pathlength, which had to be filled and emptied by hand. Even with this labor-intensive procedure, an analysis for up to six components takes only one minute. To reduce labor and simplify the analysis, flow through cells were developed, in 1 mm and 0.5 mm pathlength. The operator places an input tube from a peristaltic pump (attached to the flow-through cell) into a container of cane juice to obtain a sample. Speed and efficiency were increased, but it was found that high mud levels in juices blocked the cell.

The Beverage Analyzer was developed for the beer industry and applied to sugarcane juice. Results on use of the Beverage Analyzer (now Juice Analyzer) over the 1995-96 sugarcane crop season in Louisiana are reported here. The NIRSystems 5665 Beverage Analyzer instrument (liquid samples only; 1100-2500 nm, transmittance mode) was installed in the Juice Quality Laboratory, Ardoyne Farm, of the USDA-ARS Sugarcane Research Unit, Houma, Louisiana. The Beverage Analyzer was run on unfiltered and unclarified juice in parallel with the regular laboratory procedures for pol (589 nm) (1) using juice clarified with a mixture of aluminum chlorohydrate, bentonite, and calcium hydroxide (3) and Brix (refractometric) (1) on 1,800 samples. Results from the first few weeks of the crop are reported herein.

SENSITIVITY ANALYSIS OF LABORATORY METHODS

Four sets, of five samples in replicate but not labeled as such, were submitted to standard laboratory analysis, to determine standard lab error. Results on the sensitivity test at the Juice Quality Laboratory, Ardoyne Farm, are shown in Table 1. The coefficients of variation for both Brix and pol were excellent (less than 0.2%), reflecting the training, experience, and careful operation of technicians at the Laboratory.

BEVERAGE ANALYZER

The Beverage Analyzer can be used for analysis of liquid samples only: juices (cane and beet); syrups, molasses and liquors (diluted 1:1, as for standard tests).

Ease of operation of the Beverage Analyzer was a major reason for acceptability of this new method. The procedure for running samples was as follows: 1) the sample (60-80 ml) was placed in a plastic beaker on the platform of the Analyzer; 2) the two fixed probes were immersed in the sample, and the sample compartment door was closed; and 3) the spectrum was run (one key press) and after approximately 30 seconds the results for Brix and pol were printed.

Pol % juice values, read directly in the standard laboratory method, were corrected from the Schmitz equation to adjust for sucrose solids content, Brix equivalent value. Calibrations for Brix are shown in Figure 1, and for pol in Figure 2. Correlations and the standard error of calibration used for calibration and correlations and standard error of prediction used for validation are shown for Brix and pol in Table 2.

CONCLUSION

NIR analysis by the Beverage Analyzer proved acceptable for analyzing large numbers of samples. Its accuracy was comparable to the standard laboratory methods for determining both Brix and pol. Precision for pol readings was similar to the Laboratory's normal precision (excellent) and in the same order of magnitude for Brix as the laboratory's normal measurements.

The NIR Beverage Analyzer is fast. It provides rapid analysis without use of any chemicals or clarification agents, thereby qualifying as an environmentally friendly method.

The NIR Beverage Analyzer requires less labor than conventional methods. One instrument operator can run 60 samples each hour, almost 500 samples in an 8 hour shift, without assistance.

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ACKNOWLEDGMENTS

The authors gratefully acknowledge Chris Finger for summarizing the data, and the laboratory staff for analyzing the juice samples by the two methods.

Table 1. Sensitivity test at the Juice Quality Laboratory, Ardoyne Farm, showing the coefficients of variation for Brix and pol.

Analysis	Coefficient of variation
Brix	0.05%
pol	0.12%

Table 2. Calibration and validation of cane juice samples analyzed using standard and NIR methods.

Calibration			Validation	
	r^2	Std. error of cal.	r^2	Std. error of prediction
Brix	0.998	0.06	0.98	0.19
pol	0.988	0.15	0.97	0.16

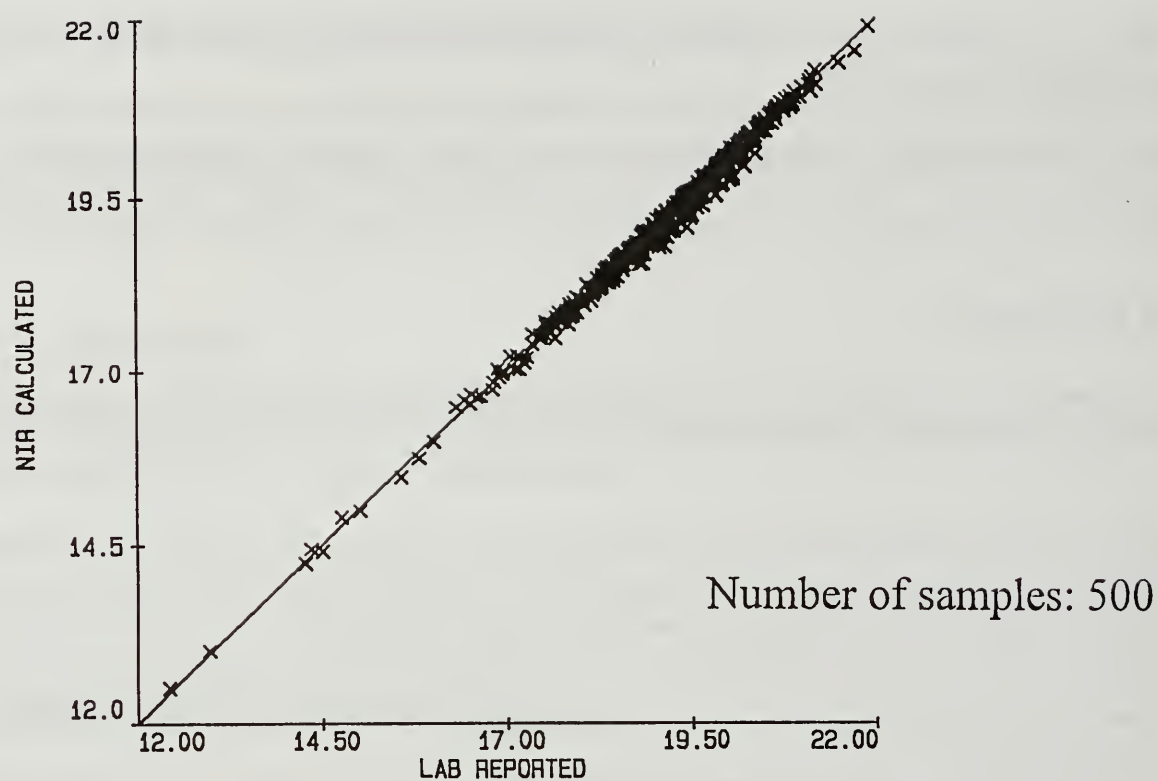


Figure1. Calibration for Brix in cane juice.

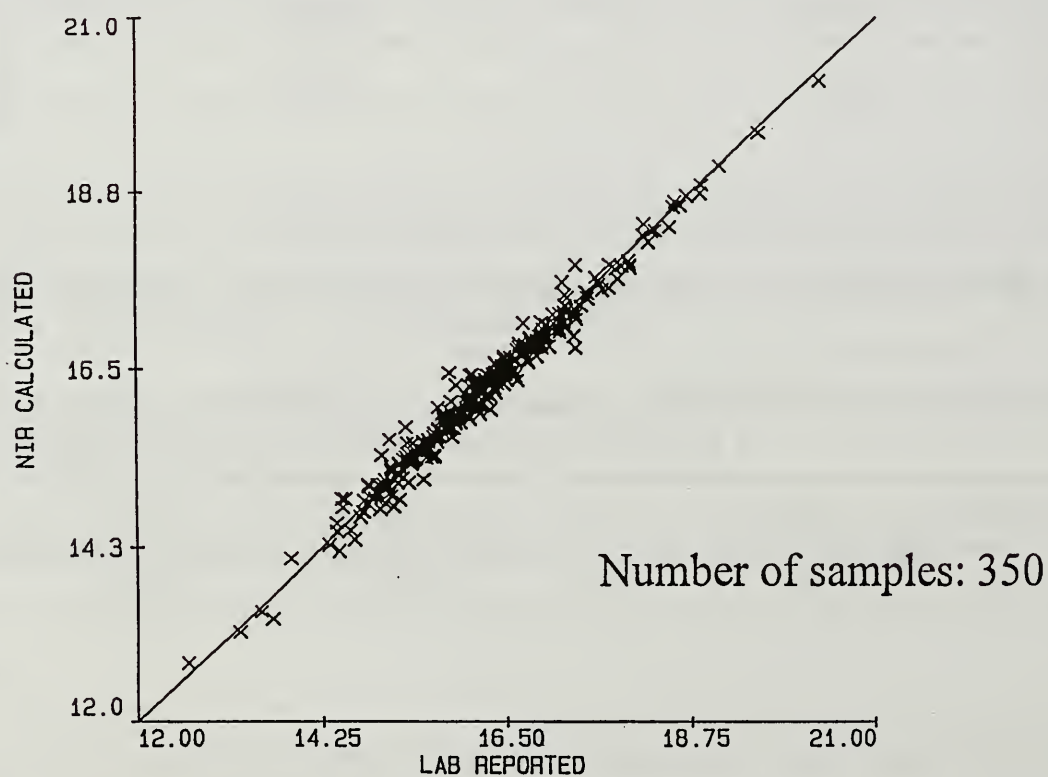


Figure 2. Calibration for pol in cane juice.

POSTER

APPLICATION OF MICROWAVE TECHNOLOGY IN THE SUGAR INDUSTRY: DIFFUSION AND CRYSTALLIZATION

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ABSTRACT

On the basis of the microwave thermal effect, microwave heating technology was used in the sugar industry to improve the diffusion and the crystallization of sucrose. It was illustrated in the experiments that the extraction of sugar juice can be greatly improved by microwave owing to the good denaturation of cane tissues, and the growth rate of sucrose crystal can also be increased by microwave irradiation because of the modification of heat transfer and crystal structure. The analysis time for sucrose content in bagasse can be reduced to a greater extent if the mixture of bagasse and the hot water are treated with microwave.

INTRODUCTION

Microwave, a kind of electromagnetic wave with frequency region between 300MHz and 300GHz, has been widely used in communication, medicine, ceramic and chemical engineering as well as the food industry. Microwave heating technology is characterized by the selective thermal effect for polar molecules (e.g. water) and quick volumetric heating which are quite feasible for the process with heat and mass transfer.

For the sugar industry, it is necessary in the juice extraction to denature the cane or beet tissues to increase the permeability of the plant cells. Mechanical treatment and thermal penetration are now the main successful denaturation methods. In spite of that, other methods are also investigated for better improvement of the denaturation. Moreover, the high viscosity of supersaturated sugar solution, resulting from greater solubility of sucrose in water, is one of the most important factors affecting the growth of sucrose crystals. Therefore, new approaches are needed in order to enhance the growth.

The aim of this paper is to examine experimentally the feasibility for the application of microwave thermal technology in the diffusion and crystallization of sucrose, based on its special intrinsic properties.

IMPROVEMENT OF SUGAR JUICE EXTRACTION BY MICROWAVE

Sugarcane contains not only sucrose but also numerous other dissolved substances, as well as cellulose or woody fibre. Water, a typical polar molecular and existing within sugarcane cells, takes an important part in the microwave thermal effects. When cane cells are subjected to microwave irradiation, the quick heating of the water component will lead to the microstructural change of the cells due to the coagulation of the protein in protoplasm and plasma membrane. Figure 1 shows the cell structure microphotograph for the

cane diffused in the environment of microwave irradiation, comparing with that not treated (Figure 2) and that diffused with the conventional hot method (Figure 3). They illustrate that microwave can not only make the cytoplasm contract, being similar to the conventional hot method, but also make the cell wall rupture. As a result, sugar diffusion in cane might be greatly improved.

The comparison was conducted for both diffusions of sucrose in cane pieces - water system (weight ratio for cane and water was 1:1) with microwave-assisted and conventional hot methods under same 750W electric power dissipation. The experimental results are shown in Figure 4.

Obviously, the extraction rate of sugar juice by microwave-assisted method is much faster than that by conventional hot method because of the microwave denaturation of cane cells and the increase of the diffusion coefficient. The mathematical models for the relationship between sucrose concentration in the juice and diffusion time with these two methods were also established as follows:

$$\ln(13.40 - 4c) = 2.60 - 0.680 t \quad (\text{Microwave-assisted extraction})$$

$$\ln(13.76 - 4c) = 2.62 - 0.071 t \quad (\text{Conventional hot extraction})$$

The influence of other factors on the extraction of sugar juice will be further studied systematically.

ENHANCEMENT OF SUCROSE CRYSTAL GROWTH BY MICROWAVE

So far as the viscous supersaturated sugar solution is concerned, there is much resistance in both heat and mass transfer during sucrose crystal growth. Existing vacuum-evaporative crystallization is of no advantage to the enhancement of crystal growth because such problems as seriously uneven temperature and concentration distribution in mother liquor and lower heat transfer rate are always included although better CV and MA mass crystals can be industrially produced with enough resident time, agitation and screening, etc. If microwave thermal technology is used in the sucrose crystallization, its quick volumetric heating characteristics might solve the problems.

The sucrose crystal growth during microwave irradiation was studied in the laboratory. The linear growth rates in the direction of the b and c axes were measured and compared with that by conventional heating under the same crystallization conditions. Figure 5 gives the experimental results and the mathematical models for the crystal growth kinetics were built in the following (where r is the supersaturation ratio):

$G_b(\mu\text{m}/\text{min}) = 5.49 \times 10^4 (r-1)^{2.272}$	(with microwave heating)
$G_b(\mu\text{m}/\text{min}) = 1.30 \times 10^4 (r-1)^{1.992}$	(with microwave heating)
$G_b(\mu\text{m}/\text{min}) = 2.43 \times 10^3 (r-1)^{1.522}$	(with conventional heating)
$G_b(\mu\text{m}/\text{min}) = 1.30 \times 10^3 (r-1)^{1.476}$	(with conventional heating)

It indicated that microwave irradiation could strengthen the sucrose crystallization. In addition to the contribution of microwave quick volumetric heating to the enhancement of the crystal growth, the structure of sucrose crystal grown during microwave irradiation was also investigated. Figure 6 gives x-ray

diffraction for both crystals grown with microwave and conventional heating respectively. There are some differences, which means that there are changes in the crystal structure.

SHORTENING OF ANALYSIS TIME FOR SUCROSE CONTENT IN BAGASSE

The analysis of sucrose content in bagasse is important in the milling management in order to obtain high sucrose extraction. In the current analysis method recommended by ICUMSA, 90 minutes are needed for the sugar extraction of 100.0g bagasse with 1000g hot water containing 5ml 12.5°Bx Na_2CO_3 solution in a boiling water bath. Due to the low analytical efficiency in the current method and the high diffusion efficiency of sucrose from cane cells assisted with microwave heating, the microwave technology was used to modify the current analysis method, i.e., the sugar extraction in the mixture of bagasse and hot water is not conducted in a boiling water bath but in a microwave oven.

Variation of sucrose content in bagasse with extraction time was measured for the microwave-modified method. The experimental results are shown in Table 1, including the result using the current method.

It can be seen in Table 1 that when microwave heating was used in the analysis of sucrose content in bagasse, it took only 25 minutes. That shows that the analytical efficiency for sucrose content in bagasse analysis could be greatly increased.

In order to check the reliability of the microwave-modified method, four samples of different bagasse were used to measure the sucrose content by microwave heating (25 min.) and by the current method (90 min.).

Table 2 shows the results. It is evident that the analytical results are reliable, that is to say, the treating time for bagasse extraction can be decreased from 90 min. to 25 min.

CONCLUSION

Microwave, as a novel heating technology, can be applied in the sugar industry for the improvement of sugar juice extraction, the enhancement of sucrose crystal growth and shortening of the analysis time for sucrose content in bagasse. Pilot plant experiments will be still required. In the future, not only should other factors affecting the process be studied in detail, but also technological consideration and economic comparison should be investigated.

ACKNOWLEDGMENT

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Table 1. Variation of the sucrose contents in bagasse with extraction time by microwave-modified method and comparison with current method.

Extraction time min	Microwave-modified method		Current method	
	°Bx (%)	Pol (%)	°Bx (%)	Pol (%)
10	7.43	6.18	/	/
15	8.05	6.70	/	/
20	9.09	7.87	/	/
25	9.14	8.21	/	/
30	9.03	8.16	/	/
35	8.86	8.09	/	/
90	/	/	8.74	8.12

Table 2. Analytic data of sucrose content in different bagasses with microwave-modified method (25 min.) and current method (90 min.).

Analysis #	Microwave-modified method		Current method	
	Bx (%)	Pol (%)	Bx (%)	Pol (%)
1	8.72	7.30	8.10	7.22
2	9.17	7.53	8.69	7.34
3	8.42	7.25	8.23	7.19
4	8.36	6.93	9.03	7.74



Figure 1. Cell structure microphotograph for cane diffused during microwave irradiation.



Figure 2. Cell structure microphotograph for fresh cane.



Figure 3. Cell structure microphotograph for cane diffused with conventional hot method.

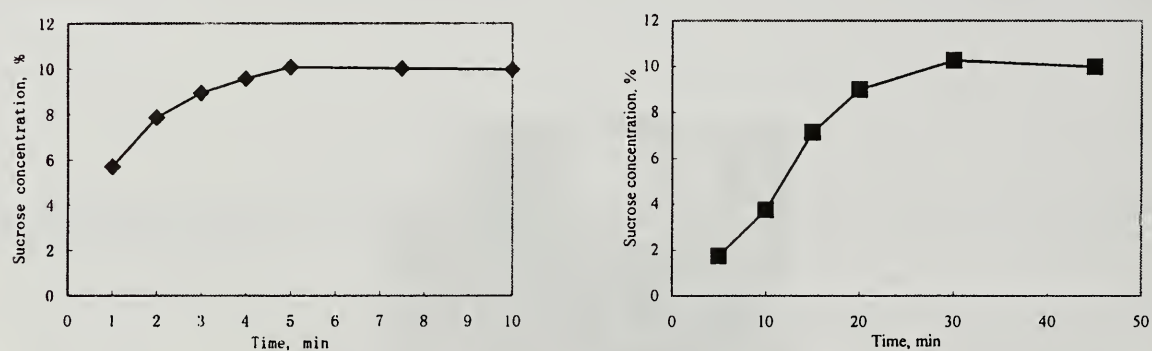


Figure 4. Sugar juice extraction with (left) microwave-assisted and (right) conventional hot methods.

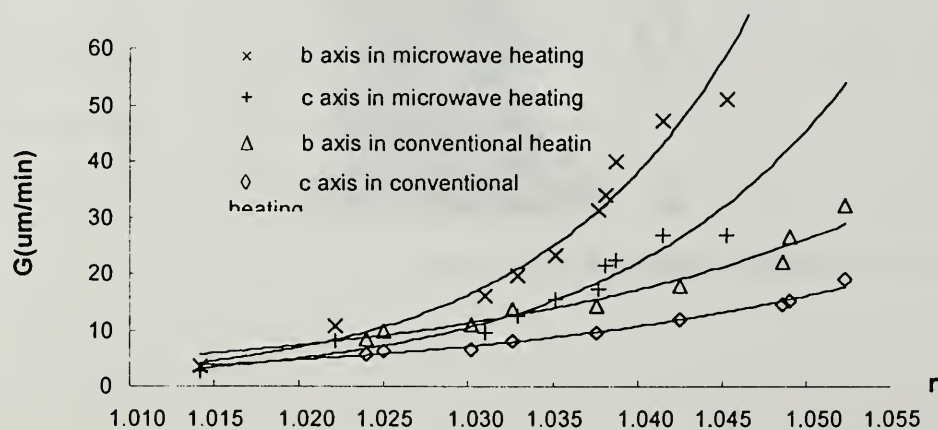


Figure 5. Growth rate of sucrose crystals during microwave and conventional heating.

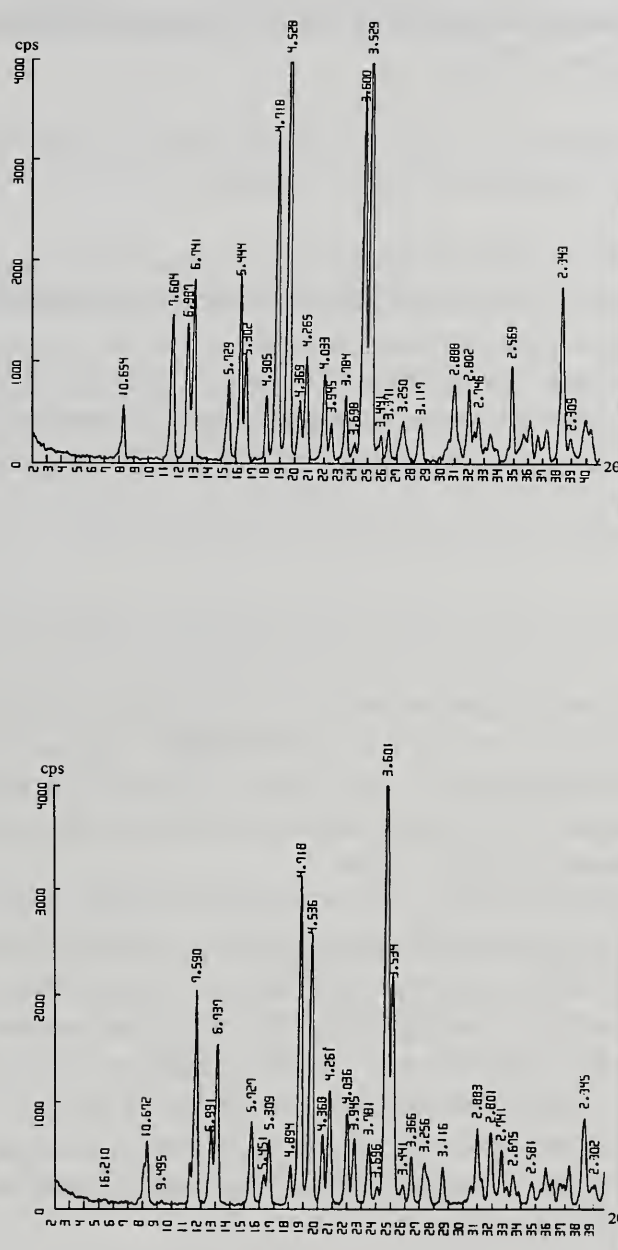


Figure 6. X-ray diffraction for both crystals grown with (top) microwave and (bottom) conventional heating.

POSTER

EFFECT OF FLUIDIZATION ON SUCROSE CRYSTALLIZATION

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ABSTRACT

Experiments were carried out in a three-phase fluidized bed. Concentration distributions of binary sucrose crystal mixtures for different operating conditions were obtained both by measuring pressure gradients and by sampling. A solid mixing (diffusion) model was developed to evaluate the solid mixing and segregation behaviors for the crystals of each size in the mixture. The results indicated that at a given liquid velocity, axial mixing extent of both larger and smaller particles increase sharply with an increase in the gas velocity, whereas decrease slightly with an increase in the liquid velocity at a given gas velocity and with increasing weight ratio.

INTRODUCTION

Crystallization is one of the last but most important processes for sugar processing. The quality of the sugar crystal product is affected directly by the operation of crystallization. Especially, the flow characteristics of massecuite will have a tremendous influence on the quality and quantity of sucrose crystal. As a result, the original homogeneous size of seeds will display a more broad size distribution because of the massecuite flow pattern and the growth dispersion of sugar crystal.

The qualified sugar crystals can be more easily produced when the crystallizer is in good circulation, i.e., the crystallizing system is basically at the condition of fluidization and crystals with different size may be mixing well owing to the irregular motion. The bigger crystals of sucrose may settle and the smaller crystals may rise to cause segregation along the axial dimension in the crystallizer. The distribution of sugar crystals in the crystallizer will depend on the interaction of these two flowing conditions. It has not only a bearing on the absorption of sucrose in mass transfer but also on the efficiency of heat transfer and the consumption of energy in the process. Therefore, the influence of fluidization on the sucrose crystallization should be investigated both in terms of theory and practice.

FLUIDIZED BED CRYSTALLIZER

A fluidized bed crystallization system, shown in Figure 1, was set up for the experiments. The main part of this system is a fluidized bed crystallizer (4) that was made from a glass column with 56mm inner diameter and 650mm height. A glass reactor (6), immersed in a water bath (7) at a constant temperature, was used for saturated sugar solution reserves. When the solution temperature was high enough that the sugar crystals in solution were dissolved completely, the solution was pumped to the bottom of fluidized

bed by a counting pump (8) through a serpentine cooler (9), then raised to the fluidized section passing a dispenser (3) packed with glass balls. Air from a compressor (1) was blasted to the bottom of fluidized bed through a gas flowmeter (2). If the temperature in the bed is constant at the saturated temperature, the seeds will neither grow nor dissolve. In order to control the crystal number and ensure the sugar solution at a constant temperature, a serpentine heater (5) was installed between the reflux outlet of fluidized bed and the reserve glass reactor (6).

Influence of such factors as air rate, the solution flow rate, crystal size, etc. on the sucrose crystal size distribution in the fluidized bed were investigated with this trial system.

MEASUREMENT OF CRYSTAL DISTRIBUTION

For convenience sake, only two sizes of sugar crystals (MA 0.85mm and 1.42mm) were selected in the experiments to mix at different weight ratios (weight of smaller crystals:weight of bigger crystals = 1:1, 1:1.2 and 1:1.4 for a constant weight of smaller crystals 100.0g). During the experiments, the crystal samples were collected at the different axial positions in the fluidized bed with a special sampling tube. The distribution of sugar crystals weight for both sizes along the height of the bed could be gotten with sieving.

Make w_1 and w_2 represent the crystal weights for both sizes at different heights of the bed and assume that the volume of the sampling tube with height h and outer diameter d is:

$$v = \frac{\pi d^2 h}{4} \quad (1)$$

and the volume, density of sugar crystal is respectively v_s , p_s , then, the partial concentrations of crystals for both sizes are:

$$c_1 = \frac{w_1}{p_s v_s} \quad (2)$$

$$c_2 = \frac{w_2}{p_s v_s} \quad (3)$$

And the total partial crystal content in the bed is:

$$e_s = c_1 + c_2 \quad (4)$$

On the other hand, under the steady state for a three-phase system of solid, liquid and gas, there is:

$$e_s + e_l + e_g = 1 \quad (5)$$

where e_l , e_g is for solution and gas contents respectively. Therefore, the static pressure gradient along axial direction is:

$$\begin{aligned} - \frac{dp}{dz} &= (e_s p_s + e_l p_l + e_g p_g)g \\ &= e_s(p_s - p_l)g - e_g(p_l - p_s)g + p_l g \end{aligned} \quad (6)$$

The total gas content could be measured by its expansion volume, in other words, it can be got from the effective height H_e , the height before and after blast H_o and H :

$$e_g = \frac{H - H_o}{H_e} \quad (7)$$

Finally, the total solid ratio e_s could be calculated from equation (6).

RESULTS AND DISCUSSION

Figures 2 and 3 show some of experimental results, representing respectively the axial profiles of sucrose crystal concentration with different crystals weight ratios, and the effect of air rates on axial diffusion coefficients with the same solution speed of 0.162cm/s.

Figure 2 shows that under a certain solution flow rate, the concentration distribution for the bigger crystals decreases along the height of the bed (from bottom to top), and increases for the smaller crystals. In Figure 3, axial diffusion coefficient D_z indicates the mixing extent for crystals. That means that the mixing extent for different size crystals is increased with the increasing of air rate, and when the air rate rises to the turbulence area, more mixing extent for crystals can be obtained. It was found that the axial diffusion coefficient D_z is slightly decreased when the solution flow speed is increased. In addition, the mixing extent becomes small as the ratio of bigger crystals is increased.

Obviously, the axial diffusion coefficient D_z is mainly related with air rate u_g , solution speed u_l and crystal weight ratio (w_1/w_2). As a result, the distribution of sugar crystal with different sizes in a three-phase fluidized bed could be described with a mixing (diffusion) model. The experimental results gave the following regression equations:

$$D_{z1} = 54.13 + 21.27u_g^2 - 9.16u_1 - 62.21\frac{w_1}{w_2}$$

$$D_{z2} = 36.69u_s^{1.87}u_1^{-0.091}\left(\frac{w_1}{w_2}\right)^{-7.66}$$

The experimental results are valuable for sugar crystallization in a fluidized bed. In the initial stage of crystal growth after seeding, the operation must be carried out with a higher air flow rate to enhance mixing and make crystals grow in unison. In the later stage, crystallization has to be operated with a lower air flow rate to reduce the mixing to get a good classification and the larger sugar crystals will settle to the bottom for drawing down.

ACKNOWLEDGMENT

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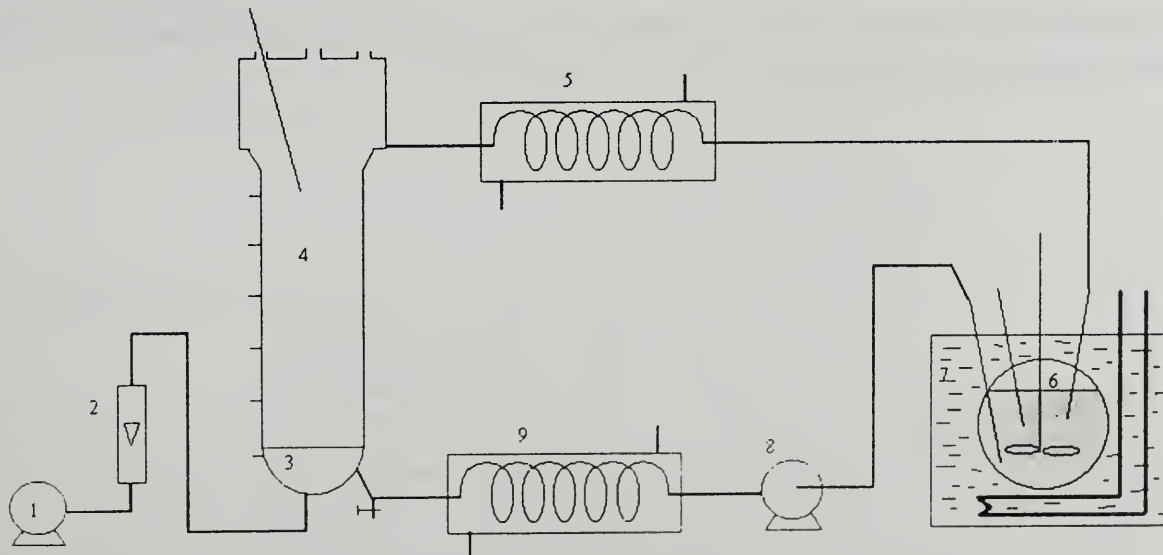


Figure 1. Experimental apparatus and flow chart of fluidized bed crystallization system.

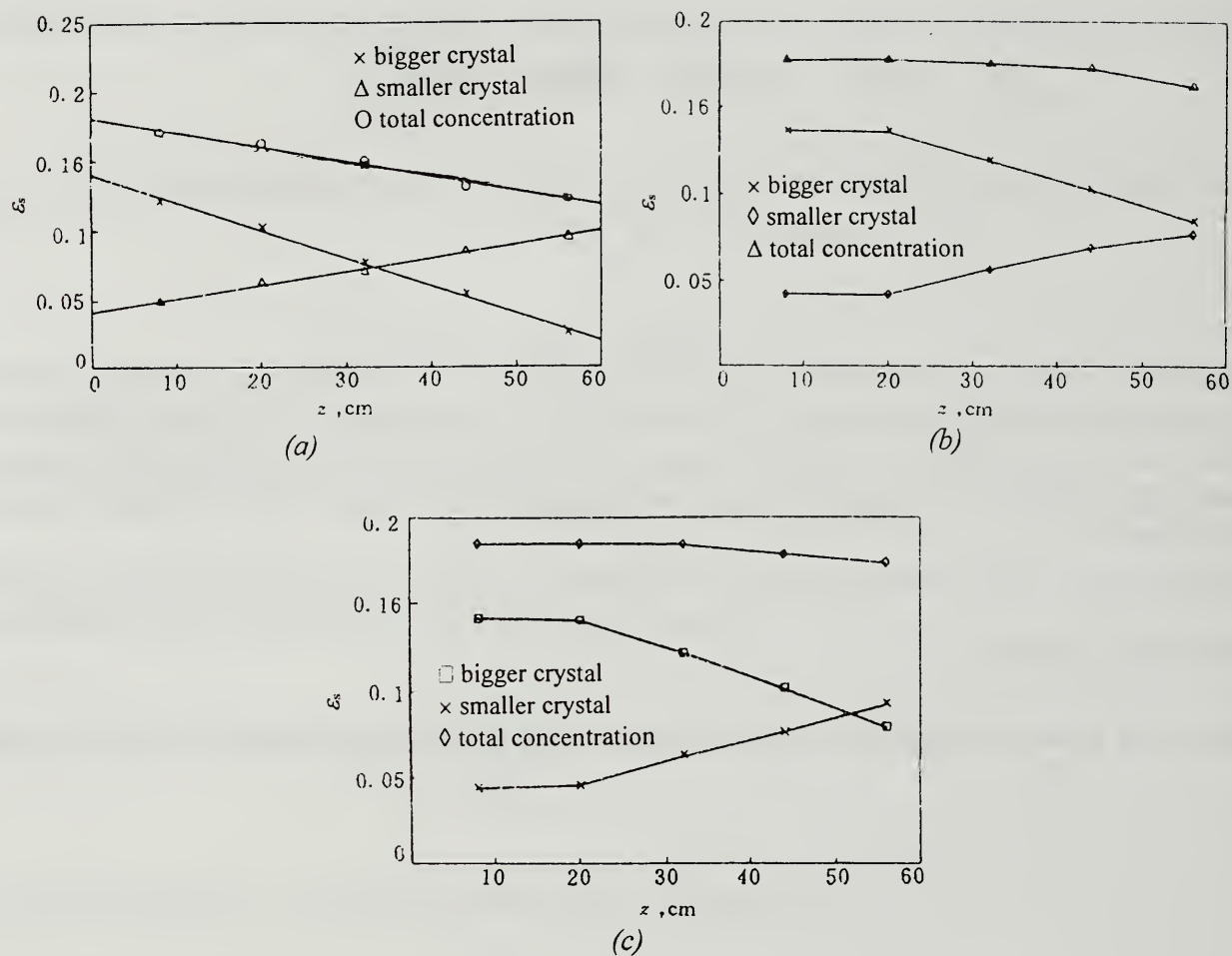


Figure 2. Axial profiles of sucrose crystals concentration with different weight ratios (the solution speed: 0.162cm/s and crystals weight ratio (a) 1:1.2, (b) 1:1.2, (c) 1:1.4).

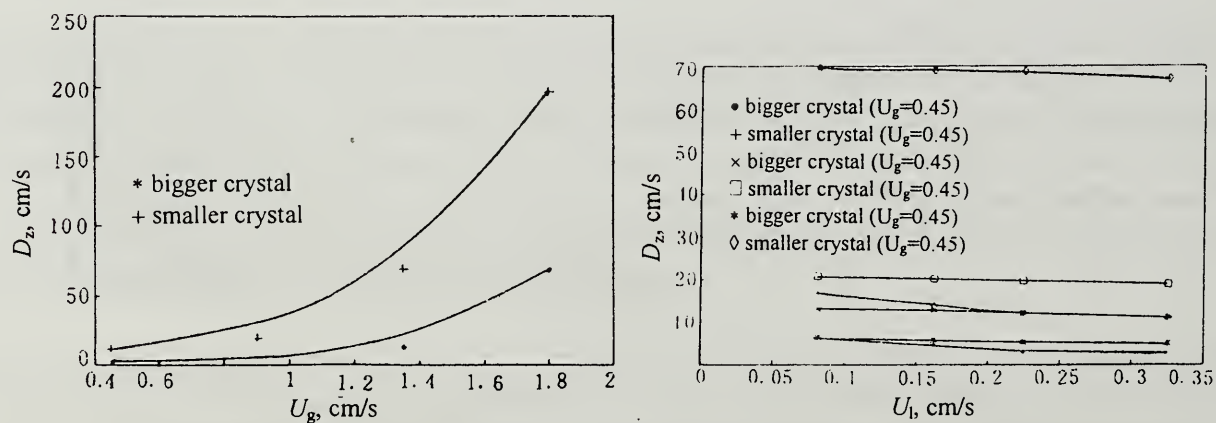


Figure 3. Effect of air and solution speeds on axial diffusion coefficients. Effect of air rate (solution speed 0.162cm/s) (left). Effect of the solution flow speed (right).

